International Journal of Veterinary Science and Medicine (2016) 4, 27–32

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International Journal of Veterinary Science and Medicine

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Short Communication

A practical guideline to remote biopsy darting of wildebeests for genetic sampling

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Received 29 June 2016; revised 6 October 2016; accepted 6 October 2016
Available online 10 December 2016

KEYWORDS

Biopsy;
Wildlife;
Dart gun;
Connochaetes taurinus;
Masai Mara;
Dan-inject

Abstract The use of biopsy darts for remote collection of tissue samples from free-ranging terrestrial and aquatic animal species has gained popularity in the recent past. The success of darting is very important since scientists may not have many chances to re-dart the same animal, especially with the free-ranging elusive wildlife species. We used wildebeest (*Connochaetes taurinus*) as a model to estimate the optimum shooting distance, pressure and the shot part of the body through which a researcher can optimize the success and amount of tissue collected from similar wild land mammalian species. Wildebeests were darted at six categories of distances ranging between 10 and 45 m and dart gun pressures of 5–14 millibar. The number of failed darts increased by increasing the darting distance: 0% (10 m), 0% (20 m), 6% (30 m), 20% (35 m), 71% (40 m), and 67% (45 m). There was a notable effect of the distances on the amount of tissue collected 20 m offered the best results. Dart gun pressure had no effect on the amount of tissue samples obtained. The amount of tissue obtained from successful darts was the same whether the animal was darted on the shoulder or thigh. In this paper, we present a practical guideline for remote biopsy darting of wildebeest to obtain optimum amount of tissue samples, which could be generalized for similar wild land mammalian species.

1. Introduction

The use of remote biopsy darts to obtain tissue samples from free-ranging wild animals has gained popularity in the recent past as it enables researchers to collect the samples without the risks and expenses of capturing live animals [1]. Commonly, tissue samples are obtained from live wild animals following chemical immobilization or physical restraints in

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Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

http://dx.doi.org/10.1016/j.ijvsm.2016.10.004
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case of small animals. Chemical immobilization is usually stressful, carries anaesthetic risks and is expensive to undertake in many wildlife species. Biopsy darting is less stressful and less risky to animals and can be an effective method for obtaining adequate amounts of tissue samples for genetic analysis. Biopsy darts have been used to collect skin tissue samples from a variety of free-ranging terrestrial and aquatic animals including African elephants, *Loxodanta Africana*, [2,3], giraffe; *Giraffa camelopardalis*, [4], bottlenose dolphins, *Tursiops truncates*, [5], polar bears, *Ursus maritimus*, [6], and killer whales, *Orcinus orca*, [7], among others.

When fired, the biopsy dart is supposed to hit the animal on preferred parts of the body with thick muscles such as the thigh, shoulder and neck. The dart is expected to cut a section of skin and underlying tissue then bounces off the animal or falls to the ground after the animal moves around. The skin tissue sample is then expected to be retained within the biopsy needle if the dart is successful [1]. Many studies have used biopsy darts [8] but none of these has evaluated the success/failure rate and the amount of the collected tissue of darts using the shooting distance, pressure and the shot part of animal's body.

Biopsy darts are usually used to collect tissue samples from the threatened animals, and hence the success of the darting is very important since scientists may not have many chances to re-dart the same animal especially with the free-ranging elusive wildlife species. The remote biopsy dart, Dan-inject, offered several advantages. A built-in CO_2 pressure gauge oriented on the dart rifle facing the shooter, along with a pressure control valve, allowed the pressure setting to be monitored and fine-tuned while aiming. In addition, a red laser sight facilitated quick accurate aiming. The bright pink color of the dart tails made the darts relatively easy to find, and the hollow rear chamber of the dart caused the darts to float tail-end-up when they fell into water

There is need to evaluate and standardize some of the existing parameters for biopsy darts in order to optimize the success and amount of tissue collected in free-ranging wild animals. In this paper, we decided to use wildebeest as a model example to estimate the optimum shooting distance, pressure and the shot part of the body for the biopsy darts. We tested three parameters, (i) the distance, (ii) pressure, and (iii) part of the body that was darted. The criteria for evaluation were (a) the failure/success in tissue collection and (b) the amount of tissue collected. Our results could be applied on other threatened land mammalian species with similar body size and behavior.

2. Materials and methods

2.1. Study area

The study was conducted in Masai Mara National Reserve (MMNR) in South-Western part of Kenya bordering Serengeti National Park (SNP) of Tanzania. The reserve is home to over 2 million migratory wildebeests and 3000 non-migratory wildebeests. The migratory wildebeests do migrate annually from Serengeti to MMNR across Mara River during July to October every year in search of adequate pasture and water [9]. The high wildlife density and the magnificent annual migration make Masai Mara National Reserve (MMNR) one of the premier tourist attractions in Kenya [10]. Wildebeests have been considered a flagship or keystone species in the Masai Mara – Serengeti ecosystem because of their occurrence in large numbers and annual migrations within and outside the ecosystem [11,12]. Wildebeests were used during this study because of their large numbers higher chances of getting candidates to dart at various distances.

We preferred darting the thigh and shoulder parts because of adequate musculature hence higher chances of getting enough tissue and less risk of hitting vital organs.

2.2. Study design

Skin tissue biopsy samples were collected from 56 attempts to dart standing free-ranging wildebeests and one carcass in Oloolaimutiak area of Masai Mara National Reserve between April and August 2015. The exercise was meant to collect skin tissue samples for studying genetic diversity among wildebeest populations in Masai Mara ecosystem. We used 1.5 ml Dan-inject darts attached to Dan-inject biopsy needles which were fired to each wildebeest by Dan-inject® (Denmark) long range projector. Wildebeests were darted at six categories of distances; 10 m, 20 m, 30 m, 35 m, 40 m, and 45 m. The distances were estimated using the range finders or sometimes just estimated by the experienced veterinarian and rangers. Sometimes the animals moved shortly before darting thereby altering the prior distance estimates, in such cases, the distance was measured exactly from the point of darting to the vehicle. For the carcass wildebeest the distances were exact.

The pressure of the dart gun was set according to the distance of the target wildebeest from the darting vehicle. For each distance, the pressure of the dart gun was set at 2 millibars above the manufacturer's recommended pressure [13], this was to ensure that the dart hit the animal with enough force to scoop tissue biopsy and bounce back or fall of immediately. The darting pressure ranged between 5 and 14 millibar (mb) depending on the distance from the target animal, such that at 10 m (5–7 mb), 20 m (8–10 mb), 30m (11–13 mb), 35 m (12 mb), 40 m (13 mb), and 45 m (14 mb). For each distance and pressure, the wildebeest's carcass was shot 4 times: 2 times for the shoulder and 2 times for the thigh.

The dart was considered successful when it hit the right target and bounced off after darting with a piece of tissue sample inside the bore of the biopsy needle. The darts that failed to bounce off were considered unsuccessful because they could not be recovered to obtain any tissue.

From these data we estimated the best distances based on the success/failure rate.

After each darting, tissue samples were retrieved from the biopsy needles and preserved in cryovials filled with 70% ethanol. The tissues were later weighed using electronic weighing machine to evaluate the amount of tissue collected from each set of darting distance, pressure and part of the body.

2.3. Statistical analyses

General Linear Models (GLMs) was applied using R Package V.2.11.1 to analyse the data.

3. Results

Fifty-six biopsy darts were fired at standing free-ranging wildebeests and one carcass. The darted free-ranging animals trotted away for about 50 m before returning to a walking pace. Forty-seven (84%) darts were successful while the rest 9 (16%) failed. The reasons for failure were varied, 50% of the failed darts bounced off the animal without cutting through the tissues, 50% failed darts got stuck to the animals and failed to bounce off immediately as expected. The number of the failed darts increased by increasing the darting distance: 0% (10 m), 0% (20 m), 6% (30 m), 20% (35 m), 71% (40 m), and 67% (45 m), (Fig. 1). Multiple R-squared: 0.3729, Adjusted R-squared: 0.2684, F-statistic: 3.568 on 5 and 30 DF, p-value: 0.01192.

The amount of tissue obtained from successful darts was the same whether the animal was darted on the shoulder or thigh; there was no statistical difference, (Fig. 2).

There was no effect of the amount of pressure used to propel biopsy darts, the amount of tissue samples obtained at different dart gun pressures was the same. Statistically, failure/success rate of the biopsy dart did not depend on the amount of pressure applied.

There was a notable effect of the distances on the amount of tissue collected. Distances of 20 m and 30 m had more tissue than 10 m. However, 20 m was better and more reliable than 30 m. Thirty meters distance had more variation in the amounts of tissue collected at different shots, there was a wide range between the highest and lowest amounts, and hence results from 30 m were quite unpredictable as compared to 20 m, which had minimum variation on the amount of tissue collected at different shots (Fig. 3).

4. Discussion

The success rate of obtaining biopsy tissue samples from free-ranging animals using remote biopsy dart techniques is normally high but may vary by many factors such as the specific methods used, the species being sampled (size, age and behavior), the terrain, vegetation cover and experience of the researcher [13–15]. To minimize the effect of such factors we included one wildebeest carcass (and hence no effect of animal

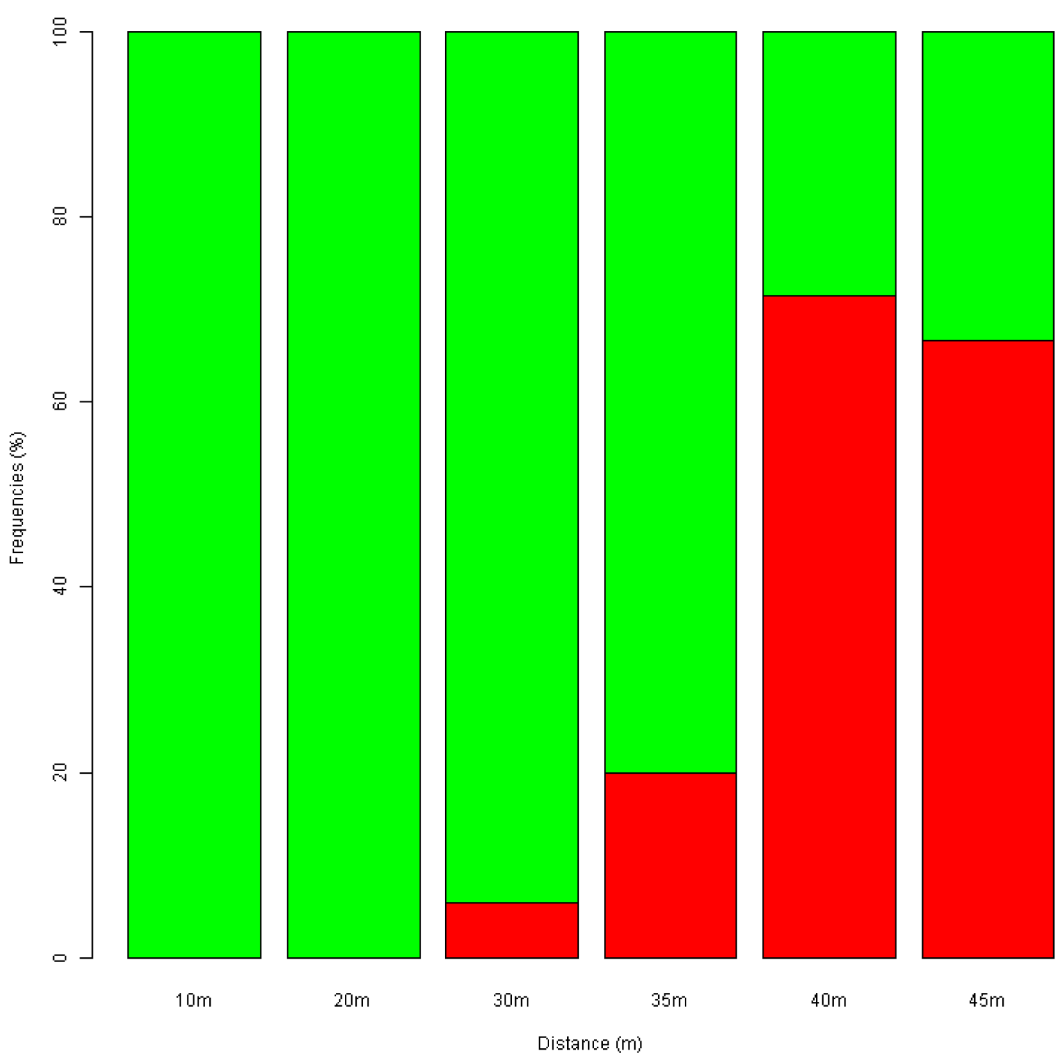

Fig. 1 Effect of distances on success or failure of biopsy darts in wildebeests (Green color: success. Red color: failure).

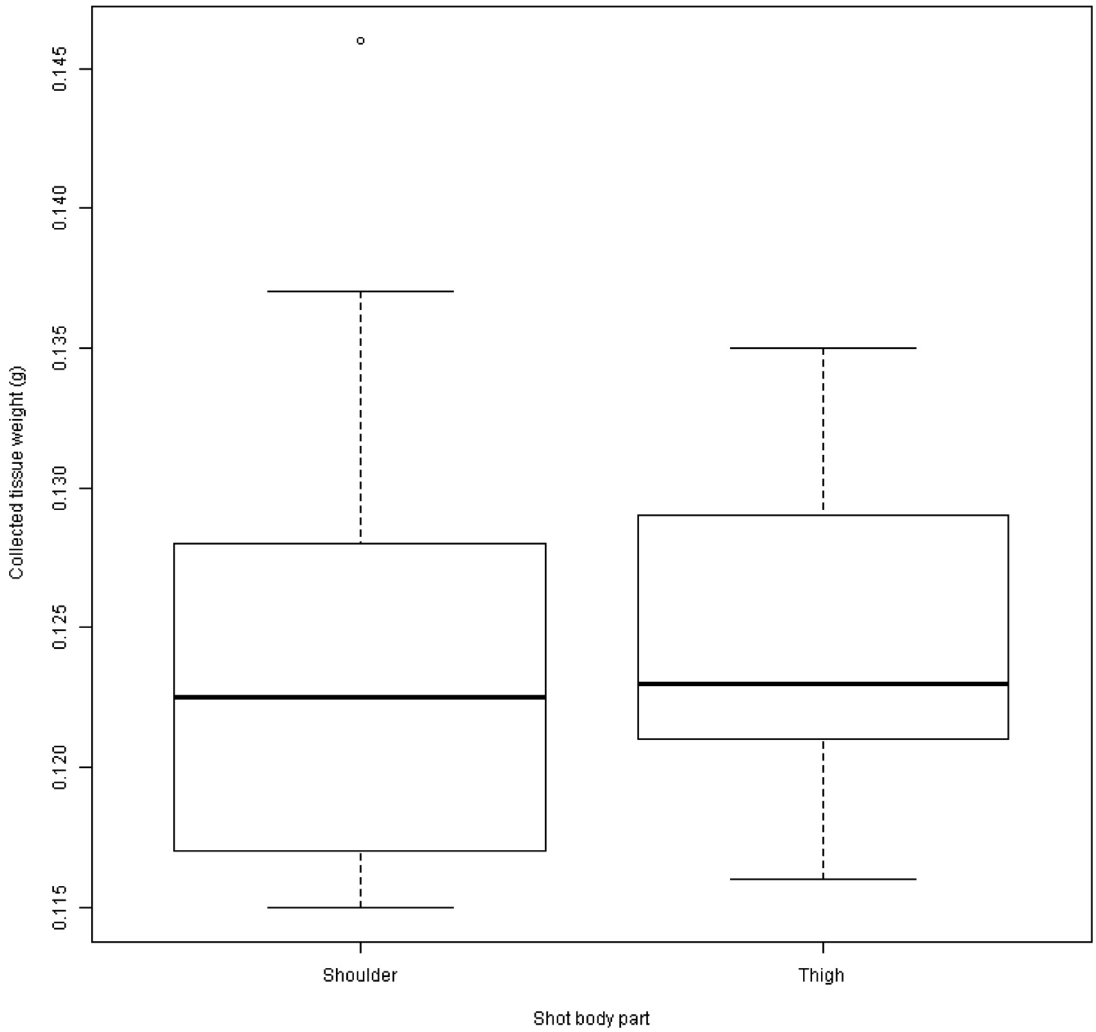

Fig. 2 Amounts of skin tissue collected from the thigh and shoulder biopsy darting targets.

size, age, behavior, terrain and vegetation cover) that we collected from the Masai river, since some of the wildebeest drown as they attempt to cross the river. This study recorded 84% success rate in obtaining biopsy tissue samples from free-ranging wildebeests. This was high compared to the findings of previous studies, on land mammalians, in which samples were obtained from 68.4% of the darts that hit small odontocetes and 84% of all darts that contacted large odontocetes and mysticetes [16]. Likewise, a system specifically designed to sample humpback whales with a pneumatic gun achieved an impressive sampling rate of 95% [17]. Oslon [13] used a similar technique to obtain biopsy tissue samples from 84% of the darted free-ranging brown bears (*Ursus arctos*) even though they were darted at closer distances of $\leqslant$15 m and differences in species behavior. Most of the published studies that have employed biopsy techniques have not focused on reporting the rate of success of acquiring biopsy samples [15].

The failed darts that bounced off from the wildebeests without cutting any tissue could be attributed to dart tip dimensions [14] or length of the biopsy needle [13]. Those darts that got stuck to the wildebeests and failed to bounce off were probably shot at acute angles [18], or they could have been fired directly perpendicular by a device that has its pressure set too high [14]. Generally, darting at long distances can lead to increased failure rates due to missed targets. However, these failure rates are commonly minimized by using high dart gun pressures. The high pressure used for darting at long distance is usually intended to provide sufficient force to propel the biopsy dart to the target and to potentially increase bounce off rate from the animal [19].

These data suggest darting distance to be 30 m or less, based on the success/failure rate (Fig. 1). In this study, 30 m was considered the optimal darting distance as it maximized the amount of tissue collected and minimized failure rate. Generally, the amount of tissue collected increased with darting distance while there were higher chances of missed the targets.

The researcher's ability to acquire a biopsy sample in free-ranging animals is usually correlated with the distance from which a dart is launched [15]. There were no missed darts in this study and this could be largely attributed to the long-term experience of the veterinarian who was darting. The terrain was open grass-land allowing proper visibility of the wildebeests and ease of movement while tracking wildebeests. The frequency of successfully hitting animals with darts increases at closer distances of less than 23 m [20], while the

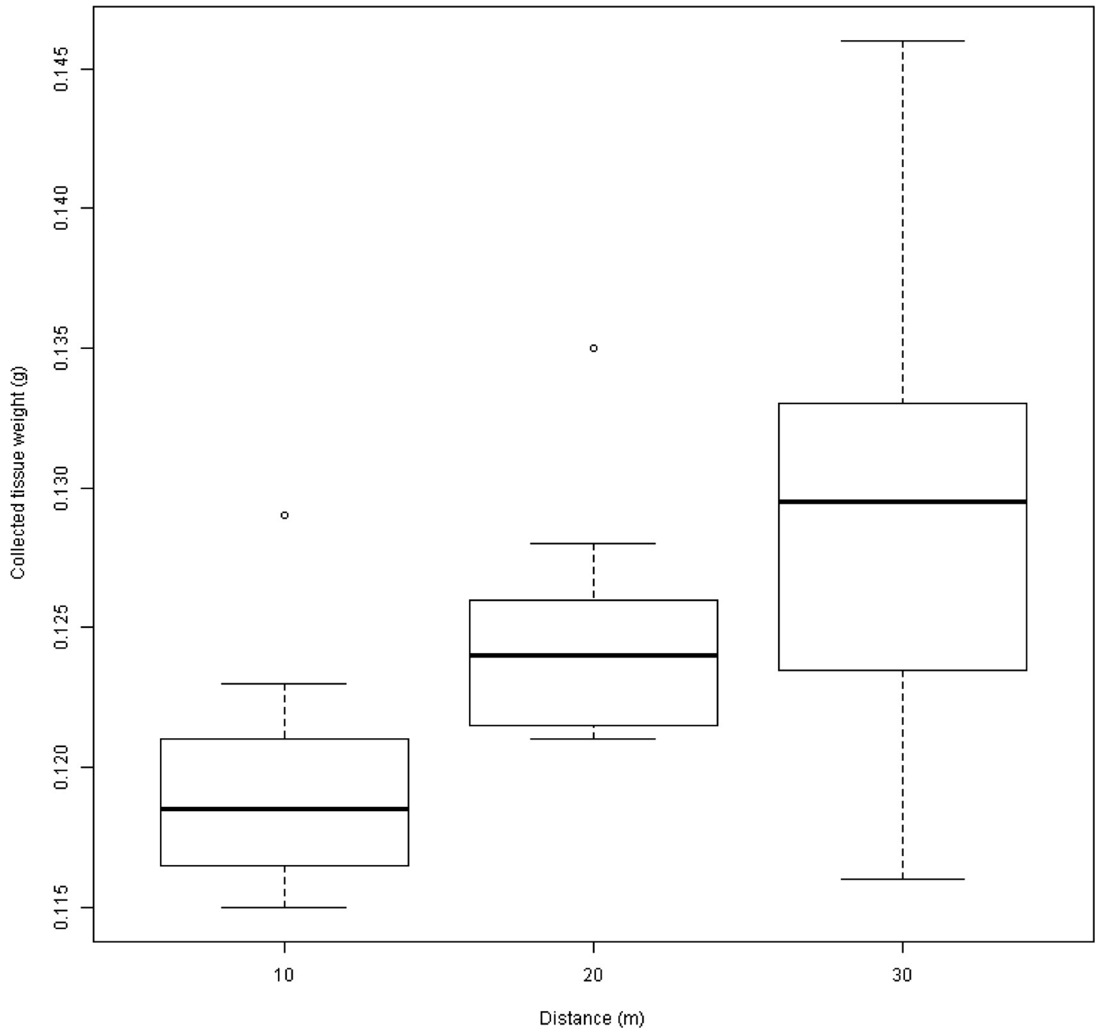

Fig. 3 Relationship between the distance of darting and amount of biopsy tissue collected.

frequency of missed darts increases with more distant firing ranges of more than 15 m [18] or more than 30 m [21]. It is important to note that definitions of "close" and "far" distances vary across species of animals to be darted [15].

There was no effect of the used pressure within each distance, and this could be attributed to the fact that the range of the pressures which can be used within each distance is limited to 6 millibars, at less pressures the dart may not reach the animal (or may bounce off with no tissue) and at high pressures the dart may injure the animals or the dart itself get damaged in the process. We used the range of pressure levels recommended by danject for each distance category, with an increase of 2mb to enable the dart to bounce off.

The ability to attain suitably large, intact samples is linked to the angle of impact as well as part of the body where the dart strikes [15]. Previous studies in whales indicate that if the dart hits high on the back where it curves toward the dorsal ridge, the dart tends to glance off with no sample or with only a minute sample of skin [18]. Our findings revealed that there was no statistical difference on the amounts of tissue collected whether the wildebeest was darted on the shoulder or thigh irrespective of the distances (Fig. 2). This was attributed to the level of experience of the darting person and darting at perpendicular angle as recommended in other studies. The probability of obtaining a sample containing both skin and blubber increases when the angle of impact is perpendicular to the body [18,22] though the angle of impact may be less critical when the dart is very sharp [18]. Having a good understanding of the target species' behavior can also increase the probability of successful biopsy sampling operations [15]. Thigh and shoulder are the most preferred body parts for collecting biopsy samples due to adequate musculature and less risk of hitting vital organs.

While darting wildebeests, we obtained higher amounts of tissue sample when the dart was launched at 20 m and 30 m as compared to darting at 10 m, which provided less amount of tissue sample. Results of little or no sample collected can also occur at very close firing ranges like 15 m [21], this explains why we were not getting adequate amount of tissue at 10 m of darting. The amount of dart gun pressure and darting equipment used may also contribute to low amount of tissue at 10 m. This could be attributed to the fact that at low distance we have to reduce the pressure of the gun, and this low pressure may cause this reduction in the collected tissue.

We obtained higher and consistent amounts of tissue samples from wildebeests while darting at 20 m distance. This should be the recommended darting distance while collecting biopsy tissue samples from free-ranging wildebeests. At longer distance of 30 m the amounts of tissue collected were highly varied and inconsistent while at 10 m there was little tissue collected.

Previous studies have recorded species variation when setting optimum distances for biopsy darts, this usually depends on the size and behavior of the animal. Biopsy samples are successfully collected from small odontocetes when darts are launched approximately 4–15 m from the target animal [5,8,23]. Yet, when biopsying larger odontocetes and mysticetes, darts are usually launched from a greater distance of approximately 5–45 m [24–27]. The actual distance from which a dart is fired is also related to the firing device used and the weather conditions [16,17].

There were no apparent adverse effects or injuries caused by biopsy darts on wildebeests except for a brief moment of jumping and running around immediately after being struck by the dart, which again confirms these type of darts as harmless and effective tool for wild animals sampling.

This study was conducted on wildebeests, and could be applied on other mammalians with similar size and behavior. Wildlife species vary in skin thickness and behavior and we therefore recommend similar studies to be conducted in other wildlife species.

References

[1] Karesh WB, Smith F, Frazier-Taylor H. A remote method for obtaining skin biopsy samples. Conserv Biol 1987;1:261–2.

[2] Muwanika VB, Siegismund HR, Okello JBA, Masembe C, Arctander P, Nyakaana S. A recent bottleneck in the warthog and elephant populations of Queen Elizabeth National Park, revealed by a comparative study of four mammalian species in Uganda National Parks. Anim Conserv 2003;6:237–45.

[3] Roca AL, Georgiadis N, Pecon-Slattery J, O'Brien SJ. Genetic evidence for two species of elephant in Africa. Science 2001;293:1473–7.

[4] Huebinger RM, Pierson DJ, De Maar TW, Brown DM, Brenneman RA, Louis EE. Characterization of 16 microsatellite marker loci in the Maasai giraffe (*Giraffa camelopardalis tippelskirchi*). Mol Ecol Notes 2002;2:531–3.

[5] Weller DW, Cockcroft VG, Würsig B, Lynn SK, Fertl D. Behavioral responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing. Aquatic Mammals 1997;23:49–58.

[6] Pagano AM, Peacock E, McKinney MA. Remote biopsy darting and marking of polar bears. Mar. Mammal Sci. 2014;30:169–83.

[7] Reisinger RR, Oosthuizen WC, Péron G, Toussaint DC, Andrews RD, de Bruyn PN. Satellite tagging and biopsy sampling of killer whales at Subantarctic Marion Island: effectiveness, immediate reactions and long-term responses. PLoS One 2014;9:e111835.

[8] Krützen M, Barré LM, Möller LM, Heithaus MR, Simms C, Sherwin WB. A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp.. Mar Mammal Sci 2002;18:863–78.

[9] Ogutu J, Owen-Smith N, Piepho HP, Said M. Continuing wildlife population declines and range contraction in the Mara region of Kenya during 1977–2009. J Zool 2011;285:99–109.

[10] Akama JS. The role of government in the development of tourism in Kenya. Int J Tourism Res 2002;4:1–14.

[11] Sinclair A, Arcese P. Serengeti past and present. Serengeti II: dynamics, management, and conservation of an ecosystem. Chicago: University of Chicago Press; 1995, p. 665.

[12] Talbot LM, Talbot MH. The wildebeest in western Masailand, East Africa. Wildl Monogr 1963;12:3–88.

[13] Olson TL. Remote biopsy dart sampling of brown bears. Katmai National Park and Preserve, PO Box 7, King Salmon, AK 99613: National Park Service, U.S. Department of Interior; 2009, p. 1–5.

[14] Best PB, Reeb D, Rew MB, Palsbøll PJ, Schaeff C, Brandão A. Biopsying southern right whales: their reactions and effects on reproduction. J Wildl Manage 2005;69:1171–80.

[15] Noren DP, Mocklin JA. Review of cetacean biopsy techniques: factors contributing to successful sample collection and physiological and behavioral impacts. Mar Mammal Sci 2012;28:154–99.

[16] Chivers SJ, Danil K, Dizon AE. Projectile biopsy sampling of cetacean species. International Whaling Commission Scientific Committee paper SC/52/O 22; 2000, p. 1–7.

[17] Lambertsen R, Baker SC, Weinrich M, Modi WS. An improved whale biopsy system designed for multidisciplinary research. In: Cristina Fossi M, Leonzio C, editors. Nondestructive biomarkers in vertebrates. Boca Raton: CRC Press; 1994. p. 219–44.

[18] Barrett-Lennard L, Smith TG, Ellis GM. A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. Mar Mammal Sci 1996;12:14–27.

[19] Dan-Inject A. Product description, biopsy needle instruction. http://www.dan-inject.com/dart_syringes.asp?klick=2. Accessed 12 Jan 2009; 2004.

[20] Jefferson TA, Hung SK. Effects of biopsy sampling on Indo-Pacific humpback dolphins (*Sousa chinensis*) in a polluted coastal environment. Aquat Mammals 2008;34:310–6.

[21] Nishiwaki S, Joyce G, Ensor P, Mermoz J, Sanpera C, Kasamatsu F. Report on the biopsy dart sampling feasibility study during the 12th IWC/IDCR Southern hemisphere minke whale assessment cruise, 1989/90; 1990, p. 22.

[22] Gauthier J, Sears R. Behavioral response of four species of balaenopterid whales to biopsy sampling. Mar Mammal Sci 1999;15:85–101.

[23] Möller LM, Beheregaray LB. Coastal bottlenose dolphins from southeastern Australia are *Tursiops aduncus* according to sequences of the mitochondrial DNA control region. Mar Mammal Sci 2001;17:249–63.

[24] Fossi MC, Marsili L, Neri G, Natoli A, Politi E, Panigada S. The use of a non-lethal tool for evaluating toxicological hazard of organochlorine contaminants in Mediterranean cetaceans: new data 10 years after the first paper published in MPB. Mar Pollut Bull 2003;46:972–82.

[25] Hooker SK, Baird RW, Al-Omari S, Gowans S, Whitehead H. Behavioral reactions of northern bottlenose whales (*Hyperoodon ampullatus*) to biopsy darting and tag attachment procedures. Fish Bull 2001;99:303–8.

[26] Ross PS, Ellis G, Ikonomou M, Barrett-Lennard L, Addison R. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. Mar Pollut Bull 2000;40:504–15.

[27] Ylitalo GM, Matkin CO, Buzitis J, Krahn MM, Jones LL, Rowles T, et al. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. Sci Total Environ 2001;281:183–203.