



Communication

Preliminary Evaluation of Potential Impacts Associated with Small Cetacean Remote Biopsy Sampling by Controlled Testing on Stranded Common Bottlenose Dolphins (*Tursiops truncatus*)

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Abstract: To explore the potential macroscopic tissue effects of select remote biopsy tools to common bottlenose dolphins (*Tursiops truncatus*), carcasses were darted and their traumatic effects on the anatomy in target and non-target areas of the body were described. In total, 87 samples were collected (target area, $n = 19$; non-target area, $n = 68$) within standardized grid partitions from five carcasses of sub-adult to adult age classes with a range of body condition scores. We broadly classified impacts penetrating completely through the blubber into muscle or deeper internal tissues as over-penetrations ($n = 51/87$, 59%). For samples collected in the defined target area, there was a low number of over-penetrations ($n = 5/51$; 10%). However, for samples collected in the defined, non-target areas, a much higher number of over-penetrations occurred ($n = 45/51$ 88%). A visual examination of some samples indicated that sample length and appearance may not be reliable guides to assess the penetration depth of the wounds. These preliminary results suggest samples collected in non-targeted areas could pose much higher risk to the individual. We encourage other researchers considering the use of remote biopsy tools to conduct similar assessments prior to field sampling to better understand the potential consequences of misplaced samples with a view towards continually improving remote biopsy tools and techniques for the benefit of cetacean welfare.

Keywords: marine mammal; remote biopsy; biological sampling; cetacean; anatomy



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1. Introduction

Remote biopsy darting has been an important data collection tool in the repertoire of marine mammal scientists [1]. Samples collected with this technique offer potential insights into the complicated group dynamics of cetacean populations, including those of endangered or infrequently encountered species. Remote biopsy data has also been used in stock management decisions in the United States (USA) for well-known species such as the common bottlenose dolphin (*Tursiops truncatus*) and a wide array of other marine mammal species in USA waters [2]. Samples collected using stable isotopes may provide insights into animal foraging ranges and prey selection [3], while blubber samples, collected for toxicology and hormone chemistry, could provide insight into the cetacean's physiological response to environmental pollutants [4,5].

Remote biopsy of marine mammals is often performed using a crossbow (or modified air rifle), and dart-delivered biopsy punch (reviewed in [1]). The majority of published remote biopsy methods describe the use of a dart-mounted biopsy punch to remove a conical plug of skin and blubber (e.g., see illustration in [6]), ideally excised at the level of the subdermal fascia at the moment of impact. The dimensions of the biopsy punch used is chosen to suit the blubber thickness of the target species. For example, for common bottlenose dolphins the biopsy punch size may range in width from 5 to 10 mm and in length from 9 to 40 mm (reviewed in [1]). To prevent penetration into the muscle tissue underlying the subdermal fascia, each remote biopsy dart is designed with a rigid backstop

that assists with dart rebound on impact, at which point the obtained sample can be collected for processing. For small cetaceans such as common bottlenose dolphins, the target area on the body described by some researchers for sample collection is high on the lateral side of the animal, just below the dorsal fin [7–10] and above the transverse processes of the vertebrae, where substantial epaxial muscles lie underneath the subdermal fascial plane [11].

Concerns for the potentially invasive aspect of this biopsy technique on smaller odontocetes like common bottlenose dolphins (hereinafter referred to as “bottlenose dolphins” or “dolphins”), have been met with published observations of post-biopsy behavior and biopsy site wound healing. While a significant number of reports suggest remote biopsy sampling has a limited behavioral and physical effect on sampled subjects [8,12–19], Noren and Mocklin [1] found a general absence of data for short and long-term effects on cetacean behavior and physiology and suggested researchers may be unable to report negative consequences. Krutzen et al. [14] noted a significant positive correlation between the size of the biopsy obtained and the strength of the dolphin’s reaction and postulated the stronger response may be due to impacts with greater penetrating force. Some strong behavioral reactions correlating to deeper penetration may indicate a hidden injury that cannot be assessed by external visual examination alone. Additionally, it is proposed that due to a variety of factors including environmental conditions and unpredictable animal behavior, samples may be inadvertently collected from non-target areas of the body.

In truth, practical and ethical challenges prevent researchers from physically examining biopsy impact sites as they are not likely to capture and immediately necropsy (or perform imaging analyses) on animals with fresh biopsy wounds. To this point some researchers have suggested that in the interest of successful sample collection and cetacean safety, all biopsy projectile systems be tested on carcasses of the desired species before sampling in the field [20]. Such testing should be done to ensure the sampling device is of appropriate power to avoid over-penetration to the target species while collecting samples of a “specified, predictable and repeatable tissue depth” [20]. Some researchers have experimented with different collection devices to compare surface wounds to the epidermis and blubber of dead animals, and to test the efficacy of their equipment [12,14,21]; however, at this time a review of the literature found no experiments evaluating the impact of a remote biopsy dart to the subdermal anatomy of any cetacean. Here, in a pilot study to explore the macroscopic tissue effects of select remote biopsy tools to common bottlenose dolphins, carcasses were darted and their traumatic effects on the anatomy in target and non-target areas of the body were described. Due to the wide variety of remote biopsy techniques and gear in use, we did not attempt to replicate all the methods researchers employ, or claim these methods represent the techniques for any specific group of researchers; we describe the results for a particular subset of remote biopsy tools and suggest improvements for future assessments of remote biopsy sampling techniques.

2. Materials and Methods

2.1. Stranded Dolphin Carcasses

Stranded common bottlenose dolphins in a state of decomposition code 2 or early code 3 [22] of adult and sub-adult age-classes [23] were collected between fall and spring seasons during 2013–2014 from Texas, USA, Gulf of Mexico beaches by the Texas Marine Mammal Stranding Network (Table 1). The carcasses were assessed for a body condition score following Mazzoil et al. [24]. All sub-adult carcasses (GA1794, GA1817, PA1031) were frozen in a -20°C freezer and thawed prior to sampling, while the two adult carcasses (GA1838, GA1846) were chilled on ice in an air-conditioned laboratory (ambient air temperature $\sim 20^{\circ}\text{C}$) until sampling commenced. This work was conducted in coordination with the Texas Marine Mammal Stranding Network under a Stranding Agreement per Section 112c of the Marine Mammal Protection Act.

Table 1. Stranding data and sampling timeline for carcasses examined in this study. Decomposition codes followed Geraci and Lounsbury [22] and body scores followed Mazzoil et al. [24].

Field ID	Sex	Length (cm)	Weight (kg)	Code	Body Score	Stranding Reported	Placed in Freezer	Sampled
GA1794	M	156	50.7	3 (early)	Ideal	4 February 2013	4 February 2013	6 September 2013
GA1817	M	167	62.8	2	Ideal	13 March 2013	13 March 2013	6 September 2013
GA1838	M	268	253.6	2	Overweight	22 November 2013	Not frozen	23 November 2013
GA1846	M	272	244.0	2	Ideal	21 December 2013	Not frozen	22 December 2013
PA1031	M	218	84.4	2	Underweight	7 March 2014	7 March 2014	25 June 2014

2.2. Carcass Sampling

Remote biopsy samples were collected from the dolphin carcasses using two different crossbows: the Barnett Panzer V (Barnett Outdoors, Tarpon Springs, FL, USA) and the MK-150A2 (Man Kung, Taichung City, Taiwan, Republic of China). The Barnett Panzer V [10,19,25], and MK-150A2 (pers. obs., Pensacola Bay August 2016; Timbalier/Terrebonne Bay June 2016) have been used to collect remote biopsy samples from common bottlenose dolphins in the field. The darts, biopsy punches, and crossbows were similar to the equipment used in the field by others [8–10,15,16,18,25,26] and evaluated by Ronje [27]. The darts were manufactured by *CETA-DART* (Aarhus, Denmark). The crossbow dart (length ~60 cm) consisted of a carbon dart shaft, a plastic nock, a 12.0 cm plastic fletching, and a threaded stainless steel flanged post enclosed in a 10.0 cm × 3.0 cm cone-shaped high-density polyurethane cast foam float (Figure 1). Each cylindrical biopsy punch (10 mm diameter × 25 mm length), threaded onto the end of the biopsy dart, featured three equidistant cylindrical barbs (approximately 4 mm in length × 2 mm diameter) brazed onto the inner surface of the punch that served to retain the sample once it was disarticulated from the target animal. In this study several of the collection darts were new while others were previously used in field work. Consequently, the foam back-stop on the previously used darts may have been compressed in comparison to new darts, which can result in variable penetration depths. Remote biopsy darts are often reused (after sterilizing) during remote biopsy sample collection projects; the darts used here were consistent with applications in the field. However, in an attempt to ensure that the length of exposed biopsy punch extending beyond the backstop was consistent, the biopsy punch was adjusted with snug-fitting rubber spacers (≤ 1 cm thick), so that no more than 25 mm of the biopsy punch was exposed. This measurement was verified before each sampling attempt.



Figure 1. Sampling ends of some darts in this study (biopsy punch not shown). A cross-section was taken of a used dart (upper), the lower dart had not yet been used for sampling. Note apparent compression of the yellow foam in the upper (previously used) dart.

Prior to sampling, all carcasses were positioned perpendicular to the sampler at table height (ventral recumbency), except for carcass (PA1031), which was placed on the floor to better simulate the angle of sampling in the field. A grid constructed of masking tape was applied to each carcass and each grid partition was assigned an alphabetical identifier (A, B, C, D, and S, Figure 2). Grid partition “S” was intended to approximate the area on the carcass targeted by other researchers in the field [7–9]. The shooting range in the laboratory was marked at 3 to 5 m from the sampler to the carcass. Biopsy collection darts were fired beginning at 5 m within each grid partition marked on the carcass, and in most cases each grid partition was sampled again at 3 or 4 m (except for PA1031, sampled only at 5 m distant). The range of 3 to 5 m for sampling was chosen because other researchers report sampling common bottlenose dolphins within a similar range and changes in dart speed and energy are negligible in that range [27]. Paper identification tags corresponding to the sample label (e.g., GA1794-A1) in each grid partition were affixed to the wound using a fishhook (Figure 3). Carcasses GA1794, GA1817, GA1838, and GA1846 were sampled on their left sides with the Barnett Panzer V (draw weight 68 kg, power stroke 31.8 cm), while carcass PA1031 was sampled on the right side with the MK-150A2 crossbow (draw-weight 68 kg, power stroke 26.7 cm), reported as slightly less powerful than the Panzer V crossbow [27]. After each event, photographs were taken of the resulting external wound, the sample collected, and the sampling end of the dart.

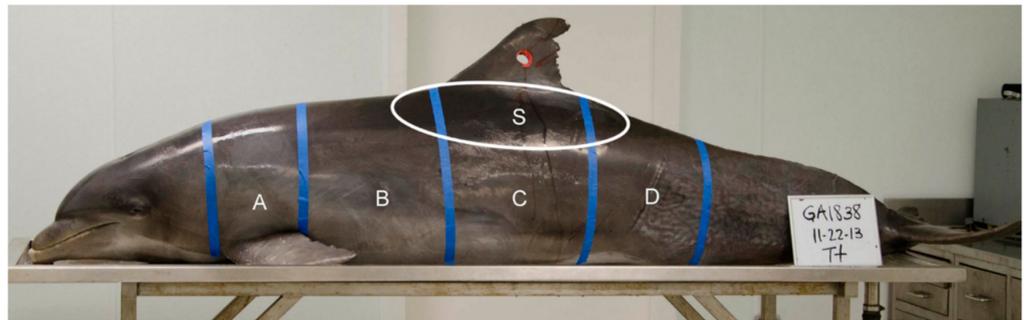


Figure 2. Grid partitions A, B, C, D, and S marked on an adult common bottlenose dolphin (GA1838) placed on a necropsy table. The hole in the dorsal fin was cut to use as a lifting point to hoist the dolphin onto the table with a crane.

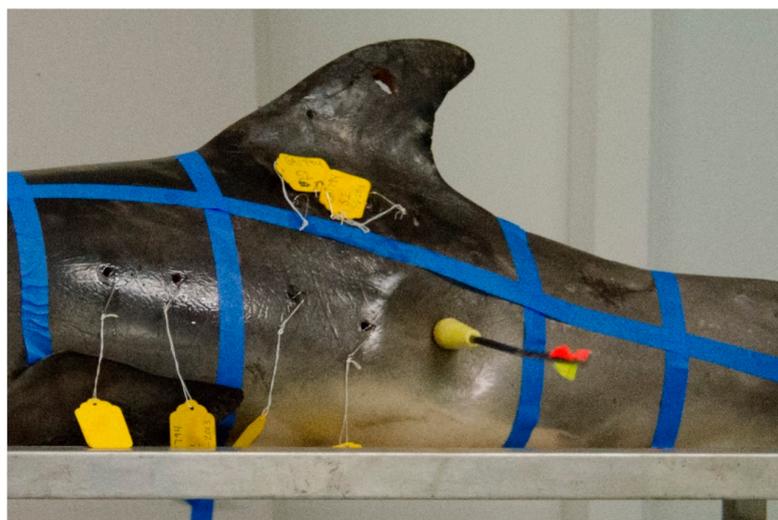


Figure 3. Remote biopsy dart impacts carcass GA1794 in grid partition C.

2.3. Lesion Examination

After sampling was completed, the blubber thickness (including skin) of each sample was measured and the examination of each carcass began with a reflection of the blubber along the subdermal fascial plane, followed by a gross examination of each wound site and a detailed description of the wound. We broadly classified impacts penetrating completely through the blubber and subdermal fascia into the muscle or other internal tissues (e.g., bone) as over-penetrations (OP). Other impacts not penetrating beyond the subdermal fascia were designated as having no visible subdermal effect (NSE) for the purpose of this study. We attempted to measure the penetration depths of each wound with a depth gauge but some of our efforts were confounded by the difficulty of precisely identifying the deepest extent of the wound. For example, if the dart did not over-penetrate beyond the firm subdermal fascia, depth measurements were relatively straightforward; however, if the biopsy dart over-penetrated into soft tissue, it was sometimes difficult to know the full extent of penetration due to the irregular surface or tearing of the tissue. In addition, samples were not always obtained at right angles due to the natural curvature of the carcass and sampling angle, resulting in some samples with uneven sides. Therefore, a statistical analysis of penetration depth measurements (and corresponding sample lengths) is not presented here.

3. Results

Carcass Sampling and Lesion Examination

In total, 87 samples were collected (target area (S), $n = 19$; non-target area (A–D), $n = 68$, Table 2, Figure 4). Overall, 59% ($n = 51/87$) of samples resulted in over-penetrations, but only 10% ($n = 5/51$) of over-penetrations were observed in the target area (S), and only in the underweight sub-adult dolphin, PA1031 (Figure 4). Only 7% ($n = 6/87$) of the samples had a blubber thickness layer ≥ 25 mm. For those samples with a blubber thickness of ≤ 25 mm, 39% were NSE. Over-penetrations were visible in 67% ($n = 6/9$) of impacts to GA1794 in grid partitions A–C. Over-penetrations were visible in 80% ($n = 8/10$) of impacts to GA1817 in grid partitions A–D, out of which three were found to impact skeletal anatomy. Over-penetrations were visible in 25% ($n = 4/16$) of impacts to GA1838 in grid partition B–D, with no visible skeletal impacts. Over-penetrations were observed in 43% ($n = 13/30$) of impacts to GA1846 in grid partitions A–D, out of which three were found to impact skeletal anatomy. Over-penetrations were observed in 91% ($n = 20/22$) of impacts to PA1031 in all grid partitions (A–S), out of which five were found to impact skeletal anatomy. For PA1031, five out of six wounds in grid partition S were classified as over-penetrations. Notable wounds in grid partitions A–D in all age-classes included biopsies and splintering of scapula and ribs. For example, impact GA1794-B1 resulted in a complete rib fracture (Figure 5). Impact GA1846-C4 resulted in a bone biopsy and fracture from the lateral and medial sides of the rib, respectively (Figure 6). An examination of the samples collected indicated their appearance was not always consistent with what would be expected based on the gross description of the wounds (Table 2). For example, samples from carcass GA1794 (A1, A2, B1, and B2), were associated with impacts to the skeleton (scapula or ribs), yet only sample A2 visually appears to have over-penetrated, and there was no visual evidence of bone fragments on the samples (Figure 7). Samples GA1794-C2 and GA1794-C3 over-penetrated into the muscle 15–20 mm beyond the subdermal fascia underlying the blubber, yet corresponding muscle tissue was not retained with the samples that would provide a visual indication of over-penetration.

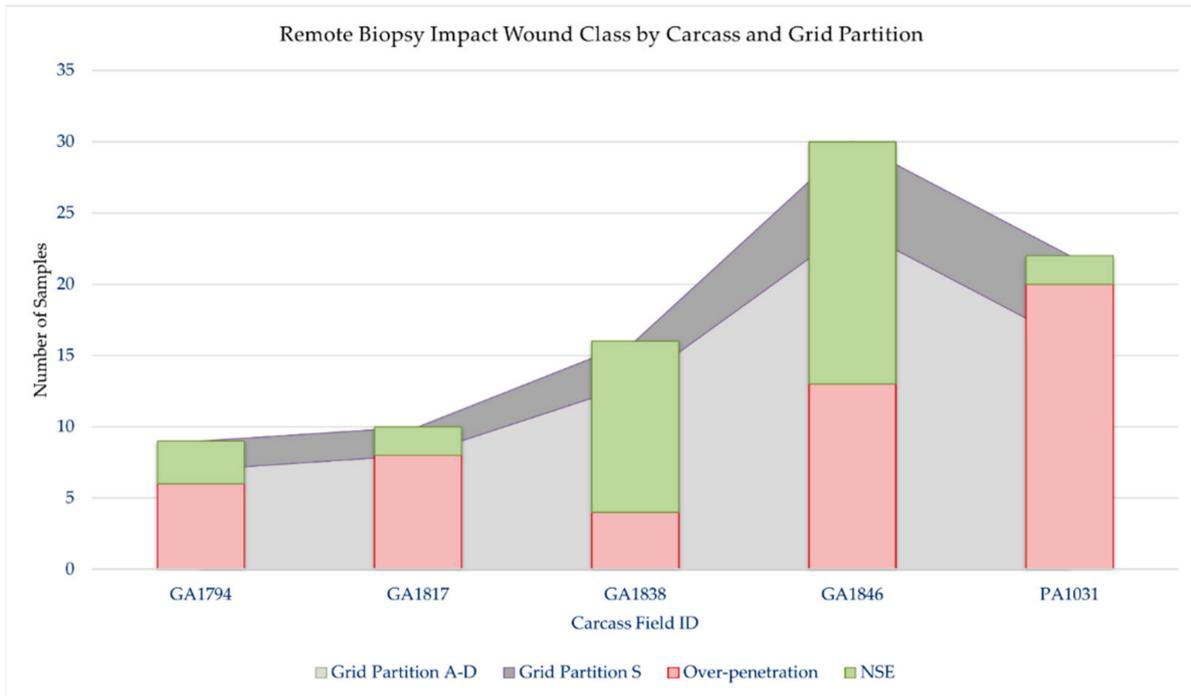


Figure 4. Remote biopsy wound classification for each carcass by grid partition. Grid partitions on each carcass were A, B, C, D, and S. Grid partitions A–D were defined as non-target areas and S was defined as the target area of each carcass. OP = over-penetration, defined as a wound penetrating through the subdermal fascia into muscle tissue; NSE = no visible subdermal effect, defined as a wound with no over-penetration.

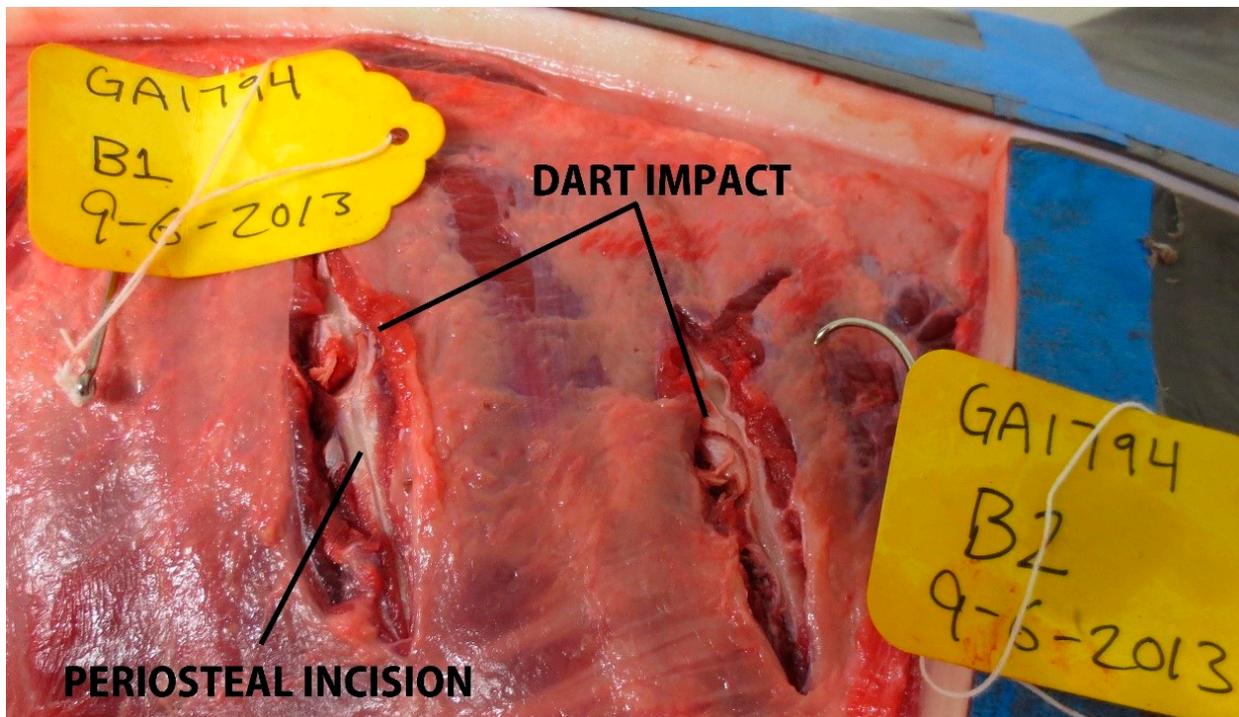


Figure 5. Rib impacts to GA1794 (subadult), with a complete rib fracture for impact GA1794-B1.

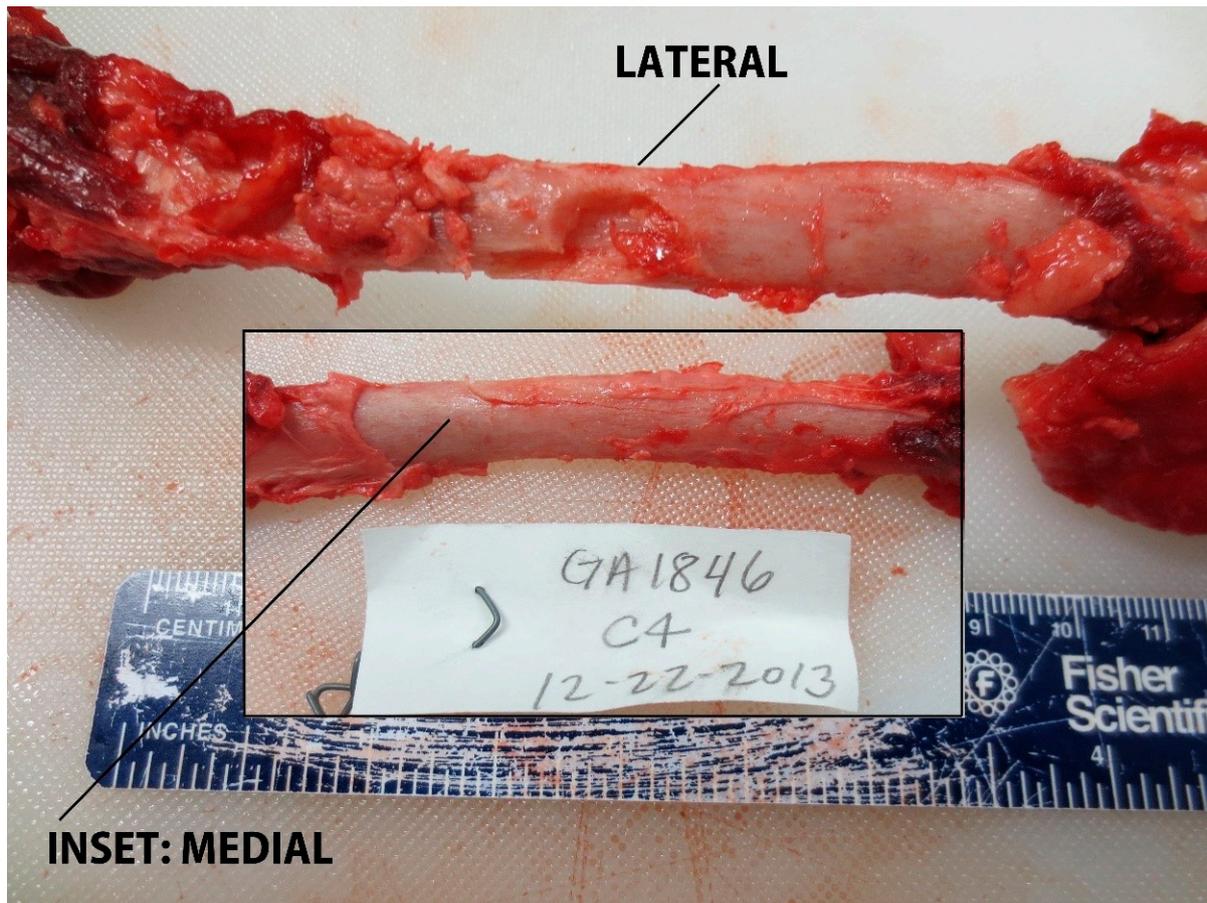


Figure 6. Rib impact in grid partition C on GA1846 (adult) with fragmentation and linear scoring of cortex on the lateral side of the rib, and a fracture apparent on the medial side of the rib.

Table 2. Impact label, wound classification (NSE = no visible subdermal effect, OP = over-penetration), sample blubber thickness, and gross description of wound sites. Unless otherwise stated, the biopsy impact created a well circumscribed full-thickness biopsy of the skin and blubber layer. Penetration depths mentioned in the gross description were measured from the dissection plane (inner surface of blubber layer), not from the external surface of the wound, and do not include measurements of the full thickness penetration of the skin/blubber. Measurements are approximate due to the irregular tearing of soft tissue.

Impact ID	Wound Class	Sample Blubber Thickness	Gross Description
GA1794-A1	OP	15	15 mm penetration of the supraspinatus muscle of scapula. Mid scapular impact. Impact created a linear dorsoventral split in the long axis of the scapula: Glenohumeral joint subchondral bone integrity compromised but cartilage layer intact.
GA1794-A2	OP	20	10 mm penetration. Cranial edge of scapula and 1/3 ventral from dorsal aspect. The edge of the scapula is splintered and irregular with a fragment imbedded in the underlying muscle belly.
GA1794-B1	OP	19	Impact on caudal aspect of 6th rib causing a semicircular defect with complete rib fracture. Rib is unstable (i.e., possible flail chest type injury)
GA1794-B2	OP	17	Impact on proximal 8th rib on cranial aspect of rib. A semicircular imprint has been left in the bone. Lesion does not penetrate pleural space. Rib integrity intact.
GA1794-C2	OP	20	15 mm penetration. Lesion is within the abdominal muscles approximately 5 cm below associated transverse process.

Table 2. Cont.

Impact ID	Wound Class	Sample Blubber Thickness	Gross Description
GA1794-C3	OP	17	20 mm penetration. Dart impact is approximately 20 mm ventral to associated transverse process. The biopsy hole is slightly oblique creating an oval shaped lesion. A small spiral pigmented epithelial tag has been left hanging within the biopsy site (possibly secondary to a direction change of dart within the blubber layer). Abdominal muscle penetration incomplete and peritoneum intact.
GA1794-D1	NSE	17	Minimal subdermal penetration (<2 mm). Approximately 15 cm ventral to associated transverse process. The blubber layer appears thicker as the lesion is near the level of the anal slit.
GA1794-S1	NSE	10	No visible penetration of the subdermal fascia
GA1794-S2	NSE	20	No visible penetration of the subdermal fascia
GA1817-A1	OP	18	12 mm penetration. Suprascapular muscle penetration. Pleural space intact. Contusion of muscle but no evidence of scapula damage.
GA1817-A2	OP	20	15 mm penetration. At dorsal border of scapula where superficial muscles thinner. Scapula penetrated with fragmentation.
GA1817-B1	OP	20	13 mm penetration. Semicircular divot into the periosteum of the cranial edge of rib #6. Rib integrity appears intact.
GA1817-B2	OP	20	Impact in center of the 9th rib, creating a shovel shaped divot that has been separated. Rib integrity appears intact.
GA1817-C1	OP	17	20 mm penetration. Caudal to last rib over abdomen. Palpation of this asymmetric lesion's depth indicates no penetration of the peritoneum
GA1817-C2	OP	17	12 mm penetration. Ventral to transverse processes.
GA1817-D1	OP	14	15 mm penetration. Penetration is approximately 30 mm ventral to transverse processes of closest vertebra and appears to penetrate the ventral spinal muscles (iliopsoas or equivalent). Peritoneum is intact.
GA1817-D2	OP	15	40 mm penetration. Lesion slightly (12 mm) above the transverse process. Impact is within a tissue/muscle plane that with gentle probing extends deep to the pedicle of the proximate vertebrae.
GA1817-S1	NSE	18	Incomplete penetration of blubber layer, however there is a peri-lesion tissue disruption (a divot) palpable from deeper layer. Impact located at crux of dorsal fin attachment to body wall.
GA1817-S2	NSE	26	Minimal impact to subdermal fascia, muscle belly has palpable impact crater but no exposed muscle fibers
GA1838-A1	NSE	14	Fascia traumatized showing redness and gelatinous appearance (carcass is relatively fresh). Location 10 mm dorsal to transverse process.
GA1838-A2	NSE	14	Ventral to A1, sub-dermal fascia traumatized but minimal effect.
GA1838-A3	NSE	10	Contusion to supraspinatus muscle—minimal effect. Impact of dart slightly askew (oblique) may have limited penetration depth.
GA1838-B1	NSE	22	Minimal observable effect.
GA1838-B2	OP	20	Oblique muscles over rib penetrated but ribs unaffected.
GA1838-B3	OP	20	Muscular band over transverse process significant in this larger animal; Penetrated but minimal effect.
GA1838-C1	NSE	19	Fascia discolored and cut from impact.
GA1838-C2	NSE	20	Slight fascial evidence of impact.
GA1838-C3	NSE	20	Slight fascial evidence of impact.
GA1838-C4	OP	21	12 mm penetration into lateral (transverse) thoracic muscle belly.

Table 2. Cont.

Impact ID	Wound Class	Sample Blubber Thickness	Gross Description
GA1838-D1	OP	20	4 mm penetration of dorsal epaxial muscle fascia (but not belly). Oblique impact secondary to body wall curvature at this level with blubber and fascia impacts slightly mis-aligned.
GA1838-D2	NSE	17	5 mm penetration into fatty fascia. Slightly oblique lesion.
GA1838-D3	OP	18–20	Penetrated 4–5 mm into muscle belly
GA1838-S1	NSE	22	Slight fascial evidence of impact, did not penetrate the fascial layer.
GA1838-S2	NSE	23	Slight oblique impact, lesion is asymmetric due to angle of body wall, does not penetrate the subcutaneous fat into the muscle.
GA1838-S3	NSE	22	Slight oblique impact, tip of probe stays within limits of blubber layer, backside does not appear discolored.
GA1846-A1	NSE	15–21	Slight fascial evidence of impact.
GA1846-A2	NSE	22–23	Dorsal caudal boarder of scapula. Fascia minimally effected.
GA1846-A3	NSE	23–24	Slight fascial evidence of impact.
GA1846-A4	OP	18	20 mm penetration over scapula, penetrated into muscle but no trauma to scapula.
GA1846-A5	OP	22	4 mm penetration. Caudal scapula bone scored (periosteal) but no cortical bone fragments apparent.
GA1846-A6	OP	26	Caudal dorsal scapula impact. Infraspinatus muscle biopsied but no trauma to scapula.
GA1846-B1	NSE	21–24	Dart has penetrated the fibrous layer covering the muscles but does not penetrate through muscle.
GA1846-B2	OP	27–28	3–4 mm penetration through the fibrous layer between blubber and dorsal epaxial muscles.
GA1846-B3	NSE	22–23	Penetrates blubber layer into thick fascia. No damage to latissimus dorsi muscle.
GA1846-B4	OP	22.5	Intercostal muscle impacted and damaged but no rib impact. Pleural space intact.
GA1846-B5	OP	26	Rib impacted with circular divot and bone splintering.
GA1846-B6	OP	22	12 mm penetration. Close to costo-chondral junction of rib and sternum. No perturbation of rib found.
GA1846-C1	OP	23	5 mm muscle penetration
GA1846-C2	NSE	21–22	Dorsal epaxial muscle superficially penetrated but minimal effect.
GA1846-C3	NSE	19–21	Pronounced fascia at this level, superficially penetrated subcutaneous layer.
GA1846-C4	OP	24	Rib impact with fragmentation and linear scoring of cortex (impact slightly to caudal edge of rib). Possible greenstick fracture.
GA1846-C5	NSE	20	Superficial muscle penetration.
GA1846-C6	OP	18	15 mm muscular penetration
GA1846-D1	NSE	21	Slight fascial evidence of impact.
GA1846-D2	OP	19–20	Muscle evident poking out from cut in fascia.
GA1846-D3	NSE	21–24	Slight fascial evidence of impact.
GA1846-D4	NSE	20–21	Slight fascial evidence of impact.
GA1846-D5	OP	17	Penetrated fascia, muscle evident poking out from cut in fascia.
GA1846-D6	OP	19	Very close to transverse process. Penetrated fascia and removed a muscle biopsy but no damage to transverse process.

Table 2. Cont.

Impact ID	Wound Class	Sample Blubber Thickness	Gross Description
GA1846-S1	NSE	15–24	Subdermal fascia not penetrated
GA1846-S2	NSE	13–21	Subdermal fascia not penetrated.
GA1846-S3	NSE	15–21	Subdermal fascia not penetrated
GA1846-S4	NSE	18–22	Incomplete penetration through the blubber layer to the subdermal fascia
GA1846-S5	NSE	13–22	Incomplete penetration through the blubber layer to the subdermal fascia
GA1846-S6	NSE	15–23	Incomplete penetration through the blubber layer to the subdermal fascia
PA1031-A1	OP	20	Divot removed from rib #3, also taken cortical bone, fragmented-linear fragment of rib taken out in addition to divot
PA1031-A2	OP	22	Perforated scapula, well circumscribed hole, little fragmenting of bone on lateral aspect of scapula, fragments on medial side of scapula
PA1031-B1	OP	27	Significant impact to top of rib head, only 1 cm tissue over rib head, impacted rib #8 and left small divot in bone, trough periosteum and into cortical bone
PA1031-B2	OP	25	Impacted caudal edge of rib #7. Semi-circular bone biopsy, no penetration into pleural space, large bone chip extends along rib on medial side, fragments of bone on medial axial component of rib. Decreased girth of rib substantially.
PA1031-B3	OP	24	Impacted rib #9 and removed divot out of rib.
PA1031-B4	OP	20–27	Hit but sample not retained, 20 mm penetration into dorsal epaxial muscle
PA1031-B5	OP	18–26	10 mm muscle penetration
PA1031-B6	OP	20	10 mm muscle penetration
PA1031-C1	OP	20	10 mm muscle penetration
PA1031-C2	NSE	19	NSE, minimal muscle penetration
PA1031-C3	OP	18	Significant damage to intercostal muscle between rib #3 and #4, ribs not impacted.
PA1031-C4	OP	19	10 mm muscular penetration, no impact to transverse process or penetration into abdominal cavity
PA1031-C5	OP	18	Sample not retained. 5 mm muscle penetration under transverse process.
PA1031-D1	OP	19	20 mm penetration of epaxial muscle
PA1031-D2	OP	20	20 mm penetration of epaxial muscle
PA1031-D3	OP	17	20 mm penetration of epaxial muscle
PA1031-S1	OP	27	20 mm penetration of epaxial muscle
PA1031-S2	OP	20	20 mm penetration of epaxial muscle
PA1031-S3	OP	18–21	10 mm penetration of epaxial muscle
PA1031-S4	OP	18–28	20 mm penetration of epaxial muscle
PA1031-S5	NSE	20	NSE, No penetration of the subdermal fascia into muscle
PA1031-S6	OP	20	15 mm muscle penetration directly over dorsal spinous process. No apparent trauma to the dorsal spinous process.



Figure 7. Example of samples obtained from each grid partition from carcass GA1794. Compare samples to the gross description of wounds in Table 2. Samples A1, A2, B1, and B2 were associated with skeletal impacts. Samples C2 and C3 over-penetrated into muscle 15 and 20 mm beyond the blubber layer, respectively. Samples D1, S1, and S3 were described as having minimal or no visible subdermal effect on the carcass.

4. Discussion

Researchers in the field must prepare for many variables influencing the trajectory and impact location of a remote biopsy dart (e.g., sea state, wind, animal behavior, platform movement, equipment malfunction, or human error). It seems feasible that out of the thousands of remote biopsy samples collected from free-ranging dolphins [1], some dart impacts have inadvertently impacted areas of the body not intended by the sampler. However, very little is reported about the potential negative effects of improperly placed dart impacts [1] if they occur. Some researchers cite examples of uncomplicated wound healing and recovery from injuries caused by intraspecific or interspecific aggression or human interactions as another reason to conclude that well-placed biopsy wounds are easily managed by the animal (e.g., [28–32]. For example, it has been suggested that wounds introduced by cookie cutter sharks (*Isistius* spp.), which typically penetrate to the subdermal fascia or underlying musculature [33] in marine mammals, may be a close

approximation of wounds incurred by a remote biopsy dart [13]. It is generally accepted that marine mammals manage cookie cutter shark wounds well [33,34], whose sequela are often nothing more than a slight depression or scar on the epidermis. However, the observation of uncomplicated wound-healing in the prey of cookie-cutter sharks may not consider the differences between the energy by which a cookie cutter shark propels itself, and the presumably significantly greater energy carried by the remote biopsy darts tested here [27].

One notable exception to the innocuous observations in support of remote biopsy was that by the Tethys Research Institute where darting resulted in the death of a common dolphin (*Delphinus delphis*) in the Eastern Ionian Sea [35]. This accidental death was thought to be the result of a complex series of factors. The dolphin in question was a 162 cm sub-adult with an unusually thin blubber layer (7 mm). This dolphin was sampled from a typical distance of 6 m with a dart calculated to carry significantly less energy than that used in this study [27,36], however the dart did not rebound and was found to penetrate deeply into the dorsal musculature, causing suspected vertebral trauma [35]. No darts were found to impact the ends of the transverse processes of the spinal vertebrae in this study, though this may be a possible scenario for bottlenose dolphins due to the limited tissue depth covering the transverse processes, and the proximity of some dart impacts (~1 cm). The post-impact skeletal damage observed here is likely due to the difference in the way fixed structures with low surface areas, like ribs, absorb energy relative to soft tissue [37]. The carcass tissue appeared to dissipate some energy to the impact area before the dart rebounds (see the impact “crater” around the biopsy dart in Figure 3), yet this energy is likely not dissipated as well if the dart impacts a more rigid part of the body (e.g., over a rib). Several instances of significant over-penetration in all animals tested here resulted from impacts in grid partition A, B, and C, but the resulting damage in those grid partitions increased for the sub-adults (GA1794 and GA1817) and underweight animal (PA1031). Damage to ribs in the sub-adult dolphins was more severe, and the results from PA1031 suggest that an animal appearing underweight should be avoided for sampling, as even well-placed shots in grid partition S (the target area) over-penetrated.

Some improvements are recommended here for future studies, and some questions remain unresolved. Although it is logistically difficult, future studies should attempt to conduct sampling and examinations on fresh carcasses, as the integrity of some tissues may be compromised after death. Mckenna et al. [38] concluded the mechanical properties of blubber and muscle did not differ significantly between their fresh and thawed specimens, which were placed into the freezer 6–8 h post-mortem and stored for one year. Their results were consistent with the CT scan results of [39] who determined that the elastic quality of *Ziphius cavirostris* connective tissue is not affected by the freeze–thaw cycle. Although the freeze–thaw cycle may not bear on our results here, there is evidence that fresh post-mortem tissues lack typical ante-mortem resilience. Mckenna et al. [38] found small differences in blubber and muscle density between post-mortem tissues and live animals. Fitzgerald and Fitzgerald [40] determined that pilot whale blubber began to lose elasticity immediately after death. Whale muscle has been shown to reasonably approximate the post-mortem changes of other mammalian muscle [41]. If stiffness in the post-rigor muscle of common bottlenose dolphins is significantly changed compared to live tissues [42], then our study may be biased toward over-penetration compared to live animal biopsies.

Our laboratory shooting range could not duplicate all field environments. Among the several complicating factors that must be considered when assessing penetration depth of each impact are dart design and energy, dimensions of the biopsy punch, blubber thickness and body condition, as well as corporeal impact angle and location. The field angle of a dart trajectory may vary with the height of the shooting platform, skill of the sampler, distance to target, animal behavior, wind, and sea state. Since an impact perpendicular to a body will translate more energy than an impact at an angle [37] it may be possible that the over-penetrations of impacts to perpendicular areas of the carcass in the lab were intensified. Thus, future studies should consider an alternative to placing the carcass at

table height. It is also possible that by placing the carcass on a solid surface (relative to a buoyant body in the water), that the carcass absorbed energy differently than a live animal in water. It is possible that the use of darts with variable amounts of foam backstop integrity resulted in more severe wounds in some cases, or that the rubber spacers used to standardize the length of exposed biopsy punch enabled a greater penetration depth (≤ 1 cm greater) if compressed upon impact; but if so, the results were consistently NSE for the target area except for the emaciated carcass (PA1031). The use of a dart with a metal backstop may be useful to minimize over-penetration as well as standardize the evaluation of dart impacts, but the corporeal location of dart impact, and dart energy, are also critical considerations. Remote biopsy projection systems using darts with less mass, and presumably less penetrating energy (e.g., “PAXARMS” [14]) may also be an alternative to consider for small cetaceans. Some researchers use biopsy punches with a length that corresponds to the seasonality and latitude of the study population (e.g., 17 mm [7]), and the blubber thickness measurements of the samples collected here seem to support that, although over-penetrations did not always occur when the blubber thickness was < 25 mm. Visual examination of samples may not accurately indicate the penetration depth or severity of the wound, because soft tissue and bone fragments may not be retained in the sample. It is recommended that equipment be tested on carcasses of the same species, age-class, region, and season as the planned biopsy collection project due to possible differences in blubber thickness and body condition. Finally, these results likely will vary from the results obtained with other dart types and projection devices; we suggest that future research approaches similar assessments and tool development collaboratively to better understand the wider spectrum of remote biopsy device, dart, biopsy punch, and species combinations, with their respective variations in power, dimensions, and anatomy.

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