Journal of Zoo and Wildlife Medicine 47(1): 275–279, 2016

VALIDATION OF ULTRASOUND AS A NONINVASIVE TOOL TO MEASURE SUBCUTANEOUS FAT DEPTH IN LEATHERBACK SEA TURTLES (*DERMOCHELYS CORIACEA*)

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Abstract: Leatherback turtles (*Dermochelys coriacea*) undergo substantial cyclical changes in body condition between foraging and nesting. Ultrasonography has been used to measure subcutaneous fat as an indicator of body condition in many species but has not been applied in sea turtles. To validate this technique in leatherback turtles, ultrasound images were obtained from 36 live-captured and dead-stranded immature and adult turtles from foraging and nesting areas in the Pacific and Atlantic oceans. Ultrasound measurements were compared with direct measurements from surgical biopsy or necropsy. Tissue architecture was confirmed histologically in a subset of turtles. The dorsal shoulder region provided the best site for differentiation of tissues. Maximum fat depth values with the front flipper in a neutral (45–90°) position demonstrated good correlation with direct measurements. Ultrasound-derived fat measurements may be used in the future for quantitative assessment of body condition as an index of health in this critically endangered species.
Key words: Body condition, *Dermochelys coriacea*, fat, health, leatherback sea turtle, ultrasound.

BRIEF COMMUNICATION

Body condition evaluation in free-ranging wildlife provides an important index for health assessment, foraging success, and reproductive success. Determination of body condition in sea turtles has been based upon subjective visual assessment, categorical ranking, and relative indices of morphometric data.[1,6] Ultrasonography has been used to measure subcutaneous fat depth as an approximation of body condition in humans, domestic animals, and wildlife; however, this method has not been developed for any sea turtle species.[5,11–15] In marine mammals, ultrasonography has documented dynamic seasonal changes in blubber depth and regional patterns of blubber deposition and mobilization.[5,13–15] Among sea turtles, leatherback turtles (*Dermochelys coriacea*) are ideal candidates in which to explore the use of ultrasonography because of their highly developed peripheral fat layers, which allow them to forage in temperate waters.[2,4,9] The leatherback's unique skin-covered carapace is lined with a dense layer of fibro-adipose blubber, which confers structure and insulation, while peripheral fat provides thermal and energetic support for fasting during long-distance migration to tropical nesting grounds.[4,7] Seasonal deposition and mobilization of fat result in substantial cyclical changes in body condition, characterized by extreme alterations in body mass, carapace shape, prominence of dorsal ridges, and thickness of the neck and base of extremities.[3,8,10] The goals of this study were 1) to validate the use of ultrasonography to accurately measure subcutaneous fat depth for the first time in a sea turtle and 2) to determine the best location from which to obtain consistent images.

Ultrasonography was performed on a total of 36 turtles: live foraging subadults and adults captured at sea from central California (n = 9) and Nova Scotia ($n = 3$); live nesting adult females

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from Florida ($n = 16$); dead-stranded adults from California ($n = 2$), Massachusetts ($n = 1$), and Nova Scotia ($n = 2$); and dead immatures recovered as fisheries bycatch from the Pacific Islands region ($n = 3$). For live turtles, ultrasound was performed on the capture boat or beach during oviposition. A portable ultrasound machine (Vet 180 Plus, SonoSite, Inc., Bothell, Washington 98021, USA) with C60/5-2 MHz broadband, 60-mm head width curvilinear transducer was used to determine tissue boundaries of the epidermis, dermis, subcutaneous fat, and underlying muscle. The transducer was positioned longitudinally, with the ultrasound beam oriented perpendicularly to the tissue. The depth was set between 4.0 and 7.5 cm, and the gain was adjusted manually. Images were captured and the machine's calipers were used to record total depth (epidermis + dermis + fat) and fat depth to the nearest 0.01 cm in the center of the image. Ultrasound images were obtained from four anatomic sites: dorsal shoulder region, lateral neck, dorsal neck, and dorsal base of the hind flipper. Ultrasound sites were chosen based upon previously identified regions of fat deposition, accessibility of the site during nesting and on a capture boat, and ability of the ultrasound signal to penetrate the tissue.[2–4,8] Rigid landmarks on the carapace were used as reference points for transducer placement. Based upon limited observations of necropsy specimens, an assumption was made that fat deposition was bilaterally symmetrical, so ultrasound was performed only on the left side of the body to minimize field sampling time.

Ultrasound measurements were confirmed grossly or histologically in a subset of turtles through direct measurement via surgical fat biopsy in live turtles ($n = 6$) or necropsy in dead turtles ($n = 8$). Total depth measurements were used as the basis for method comparison. Surgical biopsies in live-captured turtles were performed by the same veterinarian (HSH) using sterile surgical instruments, as follows: the skin was cleaned with three alternating scrubs of dilute chlorhexidine scrub and isopropyl alcohol; local anesthetic (Lidocaine HCl 2%, Sparhawk Laboratories, Inc., Lenexa, Kansas 66215, USA; 3 ml i.d.) was administered via syringe with an 18-ga, 1.5-inch needle; the skin was incised approximately 3 cm; a full-thickness fat biopsy was collected; a probe was inserted to the level of the muscle fascia for direct measurement of total depth to the nearest 0.1 cm; and the skin was sutured with 2-0 PDS in a cruciate pattern.

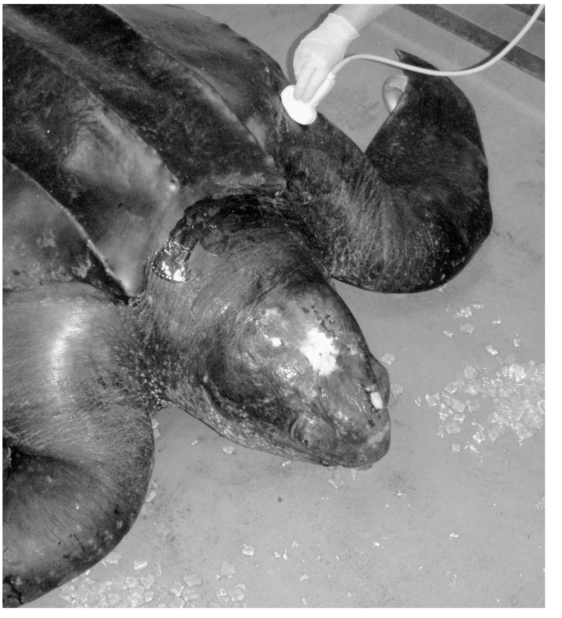

Figure 1. Dead-stranded adult male leatherback turtle from California, illustrating the location and position of the ultrasound transducer at the dorsal shoulder region. The probe is positioned perpendicular to the body and oriented longitudinally (parallel to the dorsal ridges), just cranial to the carapace margin at the level of L2 (the second dorsal ridge to the left of the midline). Note the neutral position (45–90°) of the front flippers relative to the plane of the body.

The dorsal shoulder region was determined to be the best site for ultrasound of subcutaneous fat based on gross anatomy and image quality. Preliminary observations indicated that the soft tissue of the dorsal shoulder region appeared much less affected by body position or animal movement, as compared with the neck and dorsal base of the hind flipper. The dorsal and lateral fat pads of the neck continuously stretched and swelled with head movement, particularly when turtles lifted their heads to breathe. The dorsal base of the hind flipper appeared the most variable, with the fat pad bunching up when the flipper was tucked under the turtle and flattening out when it was extended, as during oviposition. Ultrasound images obtained at the dorsal shoulder region site consistently showed clear tissue layers compared with the other sites. The transducer was positioned in a longitudinal orientation on the skin just cranial to the carapace margin at the level of L2, the second dorsal ridge to the left of the midline (Fig. 1).[3] In all images of the dorsal shoulder region, the hypoechoic subcutaneous fat layer was clearly visible, bordered superficially by

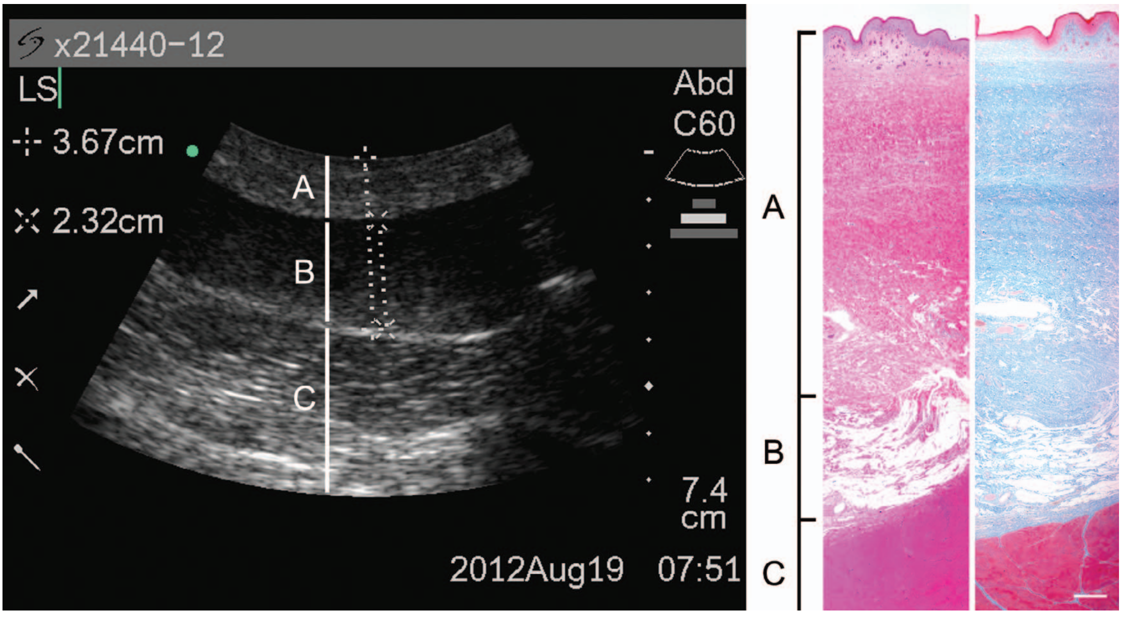

Figure 2. Ultrasound image and fat biopsy taken from the left dorsal shoulder region of two different leatherback turtles. Both turtles were emaciated dead-stranded adults from the Atlantic foraging population. A = epidermis + dermis; B = subcutaneous fat; and C = skeletal muscle. Depth of the full ultrasound image is noted in the lower right corner. Total depth (epidermis + dermis + subcutaneous fat) and fat depth are noted in the upper left corner. The corresponding histologic sections (hematoxylin and eosin on the left, Masson trichrome on the right; scale bar = 1.2 mm) illustrate the tissue architecture observed via ultrasound. The blue stain highlights the dense collagen of the dermal and adipose layers. The process of formalin fixation results in considerable tissue shrinkage, so biopsy measurements are not comparable with those obtained from fresh tissue.

a bright uniform hyperechoic band of epidermal-dermal tissue and deeply by the bright striations of the underlying muscle fascia (Fig. 2). In contrast, a distinct subcutaneous fat layer was not consistently visualized at the other three sites. It was not possible to obtain images through the carapace or lateral plastron because the tight network of dermal ossicles and lateral plastron bones prevented penetration of the ultrasound signal.

Ultrasound layers at the dorsal shoulder region were verified grossly through careful dissection of necropsy specimens, in which an incision was made to expose the tissue layers and a spinal needle (16-ga × 3.5 inches) was passed through each layer while viewing simultaneously on the ultrasound. Biopsies of the superficial soft tissue of the dorsal shoulder region provided histologic confirmation of the tissue architecture identified grossly and via ultrasound (Fig. 2). Linear regression demonstrated the strongest correlation between direct measurement and maximum ultrasound depth (Fig. 3; $r^2 = 0.72$; $P = 0.005$), as compared to mean ultrasound depth ($r^2 = 0.69$; $P = 0.007$). Ultrasound mildly underestimated total depth when compared to direct measurement.

Potential issues associated with operator error and animal position could affect the accuracy and precision of ultrasound measurements. The transducer should be oriented perpendicularly to the tissue and the calipers placed in the center of the image to avoid artifactually high measurements. Excessive manual pressure of the transducer against the skin can compress the fat, resulting in erroneously low values. To avoid this issue, sufficient coupling gel should be used to obtain a clear image and manual pressure reduced until just the weight of the transducer remains in contact with the skin. Once this issue was resolved, repeated ultrasound measurements from the same turtle obtained by two different sonographers with two different ultrasound machines varied by $\leq$0.07 cm. Ideally, a single sonographer should obtain all images to avoid interoperator variability. For direct measurements, larger incisions caused the gelatinous fat to collapse and lose its structural integrity, so care should be taken to keep incisions as small as possible. The position of the front flipper can cause alterations in fat depth measurements at the dorsal shoulder region. Multiple images obtained

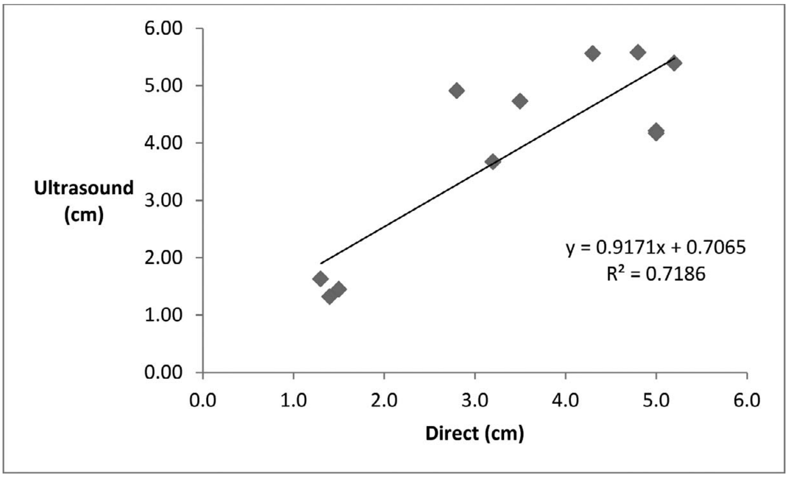

Figure 3. Comparison of total depth (epidermis + dermis + fat) at the left dorsal shoulder region obtained by ultrasound and direct measurement via biopsy or necropsy from 11 leatherback turtles. Maximum fat depth measurements via ultrasound demonstrate a good correlation with direct measurements, with ultrasound mildly underestimating total depth when compared to direct measurement.

from the same individuals showed fat depth values that were up to 1.2 cm higher when the front flippers were in a neutral position (45–90° to the plane of the body) as compared with when they were in extension (>90°) or flexion (parallel to the body), presumably as a result of relaxation of the underlying shoulder musculature in a neutral position. Since nesting leatherbacks typically hold their front flippers in a neutral position to stabilize themselves during oviposition, the same position should be maintained when obtaining ultrasound measurements on a capture boat. When multiple ultrasound measurements were available, the maximum fat depth value was found to be the most accurate. Previous trauma to the shoulder region may cause swelling or contracture of underlying tissues, as was seen in one turtle with an entanglement injury, so the contralateral side should be measured. Finally, the degree of tissue autolysis and freeze–thaw artifact could alter tissue consistency in dead animals and affect image quality.

In conclusion, portable ultrasound is a valuable noninvasive tool to measure subcutaneous fat in leatherback turtles with minimal manual restraint during nesting and at-sea capture operations. If care is taken to minimize variability in methodology and animal position, ultrasound can provide accurate measurements of peripheral fat in the dorsal shoulder region that correlate well with direct measurements. Knowledge of fat depth at this site may be useful for determination of intramuscular passive integrated transponder tag placement in the shoulder musculature, since peripheral fat may exceed the length of the delivery needle. In future studies, ultrasound-derived fat measurements may be used in conjunction with body condition scoring, morphometrics, and clinical blood data to objectively quantify body condition as an index of health in this critically endangered species.

Acknowledgments: This project was supported in part by the California Department of Fish and Wildlife's Oil Spill Response Trust Fund through the Oiled Wildlife Care Network at the Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, University of California, Davis. Field work was conducted under permits from the National Oceanic and Atmospheric Administration (NOAA) (1596-01, 1596-03, and 15634), the Florida Fish and Wildlife Conservation Commission Marine Turtle Permit (157), and Fisheries and Oceans Canada License 332697. All live animal procedures were approved by Institutional Animal Care and Use Committee (IACUC) through Moss Landing Marine Laboratories/San Jose State University Research Foundation (974). The authors thank C. Harms, M. Boor, J. Mellish, and C. Harvey-Clark for valuable scientific input; C. Fahy from the NOAA West Coast Regional Office; J. Douglas from the Moss Landing Marine Laboratories; the in-water capture and aerial teams from NOAA Southwest Fisheries Science Center and Canadian Sea Turtle Network; the necropsy teams from the California Department of Fish and Wildlife's Marine Wildlife Veterinary Care and Research Center, Atlantic Veterinary College, and NOAA Pacific Islands Fisheries Science Center, especially T. Jones; C. Innis, J. Cavin, and the New England Aquarium Departments of Animal Health and Rescue and Rehabilitation; C. Johnson and the leatherback field research team from the Loggerhead Marinelife Center; marine wildlife stranding networks in the United States and Canada; and the NOAA Pacific Islands longline fisheries observer program.

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Received for publication 2 February 2015