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Testing Detectability of PIT Tags by Size, Tagging Location, and Reader Model

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Recognizing individual sea turtles each time they are encountered is essential to determining a suite of demographic factors and to documenting behaviors such as movements. Sea turtles can be individually identified in a variety of ways, and each has advantages and disadvantages. They can be externally or internally tagged with any of an assortment of tags (Balazs 1999) or identified by distinctive physical features such as scale patterns (Schofield *et al.* 2008; Dunbar *et al.* 2014) or by genetic uniqueness (Dutton & Stewart 2013).

Passive integrated transponders (PIT tags) are commonly used as an internal tag in sea turtles (Balazs 1999). A PIT tag consists of a small, integrated circuit and antenna encased in a biocompatible glass capsule that is usually about the size of a grain of rice. This tag does not require a power source and remains dormant until activated by a signal from a reader, whereupon it transmits a unique code that is displayed by the reader. In sea turtles, a PIT tag is typically implanted into muscle just below the skin. The idea is that it is more likely to become encapsulated (and its position presumably thus stabilized) in muscle than in connective tissue (Wyneken *et al.* 2010). A PIT tag is expected to be a permanent marker (Gibbons & Andrew 2004).

When using PIT tags, sea turtle researchers must choose from many possible tagging sites and from a variety of PIT tags and PIT tag readers. The tagging site should have a suitably large muscle just below the skin that is easily located using palpation or anatomical features and that avoids delicate structures such as blood vessels, nerves, and joints. The site should be reasonably accessible for scanning with a reader, and the tag should be reliably readable at the site. Another consideration in choosing a tagging site is the probability that a tag might migrate, which could make detection of the tag less likely, or which might injure the turtle (Wyneken *et al*. 2010). In the past, the size of PIT tags required the use of a relatively large (12-gauge) implantation needle, but smaller PIT tags are now available that allow implantation with a narrower needle. This lessens the risk of injury to the turtle, minimizes pain, and reduces bleeding post-implantation. However, because the distance at which smaller PIT tags can be read is typically less than that of larger ones (Fuller *et al.* 2008), smaller PIT tags may be more difficult to read after implantation. Finally, the performance of PIT tags and readers varies, and not all readers read all PIT tags (Epperly *et al*. 2015).

We conducted the present study to provide information that might help sea turtle researchers in selecting a PIT tag and a tagging site. We also evaluated the performance of two newer models of handheld PIT tag readers that were not assessed by Epperly *et al.* (2015). We tested three null hypotheses regarding the detectability of PIT tags implanted in sea turtles: 1) there is no difference between a larger (12.5 mm) and a smaller (10.3 mm) PIT tag; 2) there is no difference among a tagging site in the shoulder, in the front flipper, and in the rear flipper; and 3) there is no difference between two newer models of handheld PIT tag readers.

The PIT tags used in this study were Biomark's ATP12 PL and MiniHPT10 (Fig. 1). The ATP12 PL is a 12.5 mm tag implanted using a 12-gauge needle. The MiniHPT10 is a 10.3 mm tag implanted using a 16-gauge (narrower than 12-gauge) needle. Both are 134.2-kHz, ISO FDX-B tags. The tagging sites that were evaluated are shown in Fig. 2. The PIT tag readers were Biomark's Global Pocket Reader Plus (GPR), an updated version of the Pocket Readers evaluated in Epperly *et al.* (2015), and the HPR Lite, which is specified as having a greater PIT tag read distance than the GPR, as well as improved durability (shockproof and waterproof).

For this study, we used the recovered carcasses of 75 green turtles (*Chelonia mydas*) killed during a cold-stunning event in the Florida panhandle during January 2018, stored frozen, and then thawed shortly before use in this study. The mean straight carapace length (SCL; measured from the nuchal notch to the posterior marginal tip) of these turtles was 34.7 cm (SE = 1.0, range = 17.4-58.0). Because PIT tag detectability might be influenced by the size of a turtle, we controlled for size by ranking the turtles into quartiles by carapace length and then by randomly assigning them in equal numbers from each quartile into one of three groups that differed in the number of PIT tags that were implanted (Table 1). A sizable group of turtles without a tag (N = 20) was used to avoid enhanced detection effort because of an assumption that few turtles lacked a tag. The tagging sites for the turtles in the tagged groups were assigned blindly before any tags were applied. For turtles assigned to receive two tags, we excluded placing tags in the four tagging site combinations that were closest to each other (left shoulder/left front flipper, right shoulder/right front flipper, both shoulders, and both rear flippers). The numbers of PIT tags implanted by tag size and tagging location are presented in Table 2. A Tyvek tag bearing the turtle's unique ID number was attached to the carcass with a plastic tie using a hole that was drilled through one of the pygal bones. For each turtle, a data sheet was completed that noted its ID number and, for each possible tagging site, whether a PIT tag had been implanted and, if so, the size of the PIT tag (10.3 mm or 12.5 mm) and the PIT tag's unique code. A 12-gauge PIT tag implantation needle was inserted through the skin at all possible tagging sites that were not used to preclude the possibility that the presence of a PIT tag in these carcasses could be ascertained by noticing a needle hole in the skin (because there would be no healing).

We recruited 40 volunteers to scan the 75 green turtle carcasses for PIT tags. Volunteers were mostly participants in the Florida Sea Turtle Stranding and Salvage Network (and so authorized to document dead, sick, or injured sea turtles in Florida). Before

Group	N	Mean straight carapace length in cm (SE, range)
No PIT tag	20	33.4 (2.0, 22.0-50.6)
One PIT tag	28	35.2 (1.8, 17.4-58.0)
Two PIT tags	27	35.1 (1.7, 25.0-54.7)

Table 1. Straight carapace lengths (measured from the nuchal notch to posterior marginal tip) of three groups of green turtle carcasses used in the present study. The groups were defined by how many PIT tags had been implanted in each carcass. There was no significant difference in SCL among the three groups, as determined by a Kruskal-Wallis ANOVA ($P = 0.685$) (Shapiro-Wilk Normality Test failed, $P < 0.05$).

participating in our study, we asked the volunteers to rank their experience in scanning sea turtles for PIT tags by choosing from among three experience categories. Five chose the high experience category (had scanned >100 turtles), 10 chose the medium experience category (had scanned 10-100 turtles), and 25 chose the low experience category (had scanned <10 turtles; some had never used a PIT-tag reader).

Volunteers participated in groups of five. Each group was first given a 10-minute scripted orientation that included a brief description of PIT tags and how they are detected; an explanation of how the work associated with this study would be conducted; and a demonstration of how to use each of the two PIT tag reader models. Each volunteer was then asked to briefly (<2 minutes) practice scanning a test PIT tag with each of the reader models. They were told to always verify that a reader was functioning properly by scanning a test PIT tag before scanning a turtle. When scanning a turtle, they were instructed to slowly move the reader over the dorsal and ventral surfaces of each flipper and shoulder in either an S-shaped or circular pattern while changing the angle of the reader in relation to the skin's surface as it was moved, always attempting to keep the head of the PIT tag reader lightly touching the turtle's skin or as close to the skin as possible. We asked them to scan the dorsal and ventral surfaces of each flipper and shoulder twice. However, if they detected a PIT tag during the first scan, they did not have to re-scan the area where that PIT tag was detected.

Each volunteer scanned turtles for PIT tags at one of five visually isolated stations (1.8 m long tables separated by partitions and each with a PIT tag reader and a test PIT tag) in a 7.6 m × 7.6 m necropsy room at the Marine Mammal Pathobiology Laboratory of the Florida Fish and Wildlife Conservation Commission (FWC). When not being used, the turtles were held in 10 wheeled trash cans (7 or 8 carcasses in each can) and kept in an adjacent cold room. To begin the work, one can with turtles was located at each station. Roving assistants placed two or three turtles at a time on the table where they would be scanned by a volunteer. When all five volunteers were ready to begin scanning a turtle, they were told to do so. After 2.5 minutes, they were asked if they were done and ready to scan the next turtle. Once everyone was ready, they were told to scan the next turtle. Once all turtles at a station had been scanned, volunteers were asked to move to an adjacent station, where they scanned the turtles at that station in the same manner. After volunteers had scanned the turtles at all five stations, they took a 15-minute break. The cans with the turtles that had been scanned were returned to the cold room and the cans with the turtles that had not yet been scanned were moved out of the cold room, one to each station, and the process was repeated. For scanning of this second group of turtles, the PIT tag reader at each station was exchanged for one that was freshly charged. Also, the PIT tag readers were deployed so that volunteers generally scanned half the turtles with one model and half the turtles with the other model and so that individual turtles were scanned with one model during some scanning sessions and with the other model during other scanning sessions. It took 4-5 hours for each group of five volunteers to scan all 75 turtles. Over a four-day period, we scheduled one group to work each morning (8 a.m.-1 p.m.) and one group to work each afternoon (1 p.m.-6 p.m.). At the end of the study, we rescanned all carcasses that had received PIT tags and verified that all tags were still present.

A proctor was assigned to assist each volunteer throughout the scanning process. The proctor moved from station to station with the volunteer and recorded the results of each scan. The volunteer would report to the proctor the turtle ID number, whether the volunteer found one or more PIT tags and, if so, the flipper in which any were found (we did not ask them to differentiate location between a front flipper and an adjacent shoulder), and the last two digits of each PIT tag's unique code. The proctor also reminded the volunteer to test the reader (by scanning a test PIT tag) before scanning each turtle. Proctors did not coach volunteers as to scanning technique and had not participated in implanting PIT tags in the carcasses, so they did not know which turtles had been tagged or at which tagging sites PIT tags were present.

Overall, the 40 volunteers had 3,280 opportunities to detect an implanted PIT tag (82 PIT tags × 40 volunteers). The results by

Tagging location	10.3 mm PIT tag (N)	12.5 mm PIT tag (N)	Total PIT tags (N)
Right shoulder	7	8	15
Left shoulder	9	9	18
Right front flipper	6	7	13
Left front flipper	10	8	18
Right rear flipper	4	4	8
Left rear flipper	5	5	10
Total	41	41	82

Table 2. The number (N) of PIT tags by tagging location and PIT tag size used in the present study. The tagging sites are shown in Figure 2.

PIT tag size, tagging location, and reader model are presented in Table 3. Volunteers detected tags during all but two opportunities (99.9%). Two volunteers each failed to detect one PIT tag while using an HPR. Both tags were 10.3 mm long, and both were in the right front flipper, but they were in different turtles. One of the volunteers who missed detecting a PIT tag was categorized as low for previous experience. The turtle for which this volunteer missed detecting a PIT tag was 33.8 cm in SCL (3rd quartile). It had a 12.5 mm tag in the left shoulder and a 10.3 mm tag in the right front flipper. This volunteer detected the 12.5 mm tag but noted that it was in the right front flipper and did not note a tag in the left front flipper. The other volunteer who missed detecting a PIT tag was categorized as high for previous experience. The turtle for which this volunteer missed detecting a tag was 23.8 cm SCL (1st quartile). It had a 10.3 mm tag in the left shoulder and another in the right front flipper. The tag in the left shoulder was detected and noted as being in the left front flipper (the nearest flipper) but no tag was noted for the right front flipper. In both cases, the failed detection attempts involved a turtle with two PIT tags, one in the left shoulder and one in the right front flipper. Because these were small turtles, those tags were relatively close to each other, and this proximity may have confounded attempts to detect both tags. However, it is of interest to note that 12 of the turtles in our study had a PIT tag in both the shoulder and in the front flipper, and yet only two of the 480 total opportunities to detect both tags in this situation failed (0.4%).

Our work indicates that at a typical implantation depth (< 1 cm below the skin) and using a standardized scanning protocol, there is no difference in detectability between the 12.5 mm and 10.3 mm PIT tags or for either of these tags among the three tagging locations, and that the two reader models work equally well. According to the product catalog provided by Biomark (www.biomark.com/technical/Biomark_product_catalog.pdf), the read distance of the 12.5 mm PIT tag is greater than that of the 10.3 mm PIT tag, and the HPR Lite can detect PIT tags from farther away than can the GPR. Nevertheless, these specifications indicate that both reader models can detect both PIT tags at a read distance as far as 11.5 cm. Maintaining contact between the reader and the turtle's skin is more difficult when scanning the contoured surfaces of the shoulder than when scanning the flat surfaces of the flippers, but even in the shoulder area, it is easy to keep either reader well within 11.5 cm of an implanted PIT tag.

The turtles used were relatively small (up to 58.0 cm), but we expect similar PIT tag detectability results even in the largest cheloniids, since PIT tags in all these turtles are implanted relatively close to the skin's surface (typically <1 cm deep). Due to a layer of subcutaneous fat in leatherbacks (*Dermochelys coriacea*), PIT tags must be implanted in these turtles as deeply as implantation needles allow (about 4 cm, McDonald and Dutton 1996), which still places the tag well within the reported read distance of both tags using either reader and when keeping the reader close to the skin. Nevertheless, to relate our findings more strongly to sea turtles >60 cm SCL and to confirm the read distances reported by the manufacturer, we determined how deeply within sea turtle tissue our two PIT tags were detectable using our two reader models. We did this by first placing five tags of each size beneath layers of foam and evaluating read distance in increments of 2.5 cm using each reader. Each tag was tested with its long axis oriented horizontally as well as vertically to the scanning surface. Once the maximum distance of detection was measured, we confirmed that these PIT tags were detectable through the same thickness of sea turtle tissue using muscle and skin collected from a dead adult green turtle. The HPR Lite and GPR consistently detected PIT tags of both sizes (and with either of the long axis orientations) at a distance through sea turtle tissue of up to 12.5 cm and 10.0 cm, respectively. The HPR Lite also consistently detected 12.5 mm tags through a thickness of sea turtle tissue of 15.0 cm. We did not detect any differences in read distance through sea turtle tissue between the two sizes of tags when using the GPR.

Our experiment was not designed to assess the effect of experience of the person scanning on the ability to effectively detect PIT tags,

Category of interest	Opportunities to detect a PIT tag		PIT tag detections (%)
PIT tag size	10.3 mm	1,640	1,638 (99.9%)
	12.5 mm	1,640	1,640 (100%)
PIT tagging location	Right shoulder	600	600 (100%)
	Left shoulder	720	720 (100%)
	Right front flipper	520	518 (99.6%)
	Left front flipper	720	720 (100%)
	Right rear flipper	320	320 (100%)
	Left rear flipper	400	400 (100%)
PIT tag reader	GPR	1,644	1,644 (100%)
	HPR	1,636	1,634 (99.9%)
Total		3,280	3,278 (99.9%)

Table 3. Results of PIT tag detection attempts by 40 volunteers who scanned for 82 PIT tags in 75 green turtle carcasses. Information on the numbers and locations of the PIT tags in these carcasses is presented in Tables 1 and 2. The PIT tags were Biomark's ATP12 PL (12.5 mm) and MiniHPT10 (10.3 mm). The tagging sites are shown in Figure 2. The PIT tag reader models were Biomark's Global Pocket Reader Plus (GPR) and HPR Lite. There was no difference in detection rates by tag size, tagging location, or reader model (binomial proportion test, $P > 0.05$).

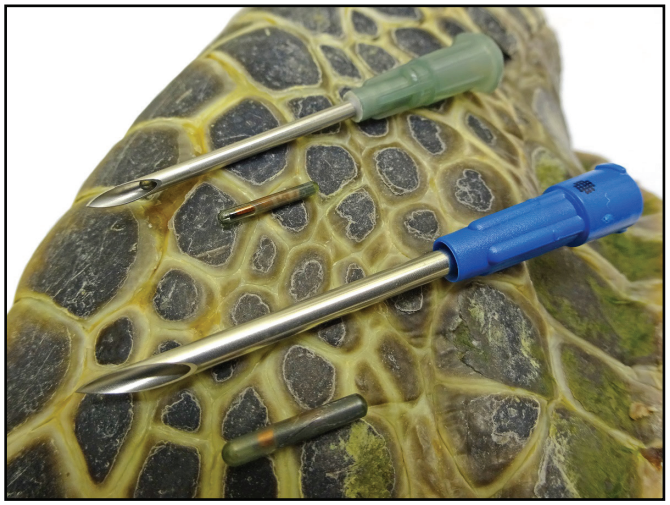

Figure 1. Relative size of PIT tags and associated implantation needles and the proximal front flipper of a juvenile green turtle (*Chelonia mydas*). Shown are a 16-gauge needle and a 10.3 mm PIT tag (MiniHPT10, top) and a 12-gauge needle and a 12.5 mm PIT tag (ATP12 PL, bottom)

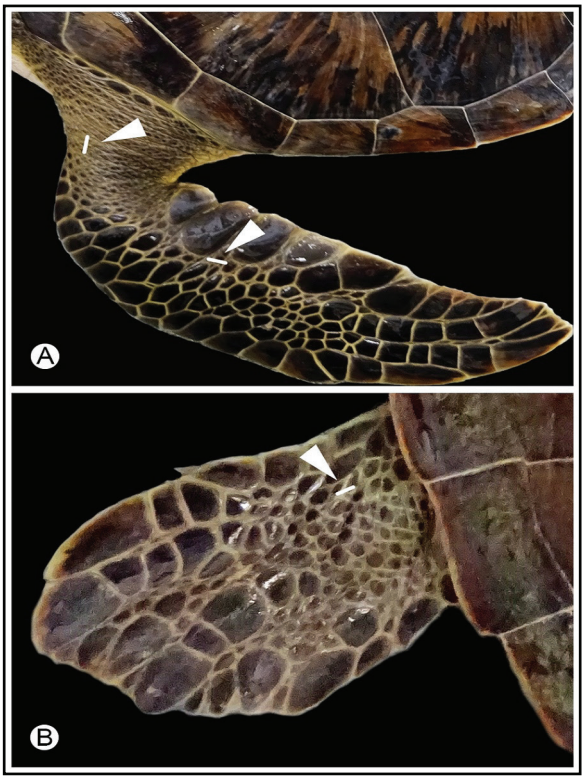

Figure 2. PIT tagging sites in the present study. At all sites, the tag is placed into muscle. The tagging sites shown in the top panel (A) are the shoulder (top marker at tip of pointer) and the front flipper (bottom marker at tip of pointer). The tagging site shown in the bottom panel (B) is the rear flipper (marker at tip of pointer).

but it was clear that even those with no experience could quickly learn to detect these tags in sea turtles with a success that equaled that of people with much experience. We believe that anyone can detect these tags if they scan using an appropriate technique such as that in the present study. We believe a proper scanning protocol is critically important but may not always be applied, even though it can be completed within a few minutes. The turtles used in this study were small- to medium-size immature turtles and while it may take longer to properly scan a larger immature or adult turtle, this will likely add only minimal time. PIT-tag scanning efforts are critical to recognizing turtles that carry PIT tags and if not conducted properly, a tag may fail to be detected and a valuable recapture opportunity would be lost. Instruction and practice in scanning for a PIT tag should be an essential component of all programs in which PIT-tagged turtles may be encountered and this training should be repeated at regular intervals to maintain good technique.

Further impetus for the current study was the observation by one of the authors (B. Stacy) that PIT tags implanted in the shoulder of live sea turtles were sometimes difficult to detect with older models of PIT tag readers, requiring a prolonged scanning effort that may not always be conducted. In our study (using carcasses), we found no difficulties in detecting PIT tags in the shoulder using the two newer models of PIT tag readers. But live sea turtles may make scanning the shoulder for PIT tags more difficult and keep the scanner farther away from the implanted tag by drawing in their front flippers and head.

Our study showed that PIT tags at each of the three tagging locations can be detected with equal success, so we cannot recommend any tagging site over the others. Which site is best for a specific sea turtle research objective will depend on other factors such as permitting conditions, the circumstances (in-water capture, nesting beach encounter) when attempting to place or read a PIT tag, individual experience and comfort with safely implanting tags at specific sites, and species- or size-dependent differences in muscle masses. We demonstrated that in small to medium-sized (immature) green turtles, a 10.3 mm PIT tag has the same detectability as a 12.5 mm PIT tag and provided evidence that this would likely be the case in larger cheloniids. We suspect the 10.3 mm PIT tag will work well in leatherbacks, but data on the efficacy of these smaller PIT tags specifically for this species are still needed. We recommend using the 10.3 mm PIT tag instead of the 12.5 mm PIT tag in all chelonids because the smaller tag has the same effective detectability but is implanted with a narrower needle, which reduces risk and discomfort for the sea turtles.

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