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Marking individual animals in wildlife studies is essential and plays a vital role in furthering our knowledge of animal populations. Mark and recapture studies delineate ranges and migratory movement patterns, help identify which populations use feeding and breeding grounds, and can contribute to vital rates where animals can be encountered across time during breeding events. Marine turtle studies also have benefited from marking of animals. External tags have long been used to mark individual nesting turtles, juvenile and adult turtles in benthic, oceanic and neritic foraging habitats (Godley et al 2003; Limpus et al. 2009; Mortimer and Carr 1987; Schmid 1998). However, some external tags have been found to cause harm or reduce the survival of an individual (Nichols et al. 1998). In addition, external tag loss is common and decreases the rate at which previously tagged individuals are identified (Balazs 1982; Bjorndal et al. 1996; Henwood 1986; Limpus 1992). Consequently, in recent years, use of Passive Integrated Transponder (PIT) tags, also termed Radio Frequency Identification tags, has increased (Balazs 1999; Broderick and Godley 1999). A PIT tag is a tiny electronic microchip encased in a glass capsule that is inserted under the skin or into muscle. It is passive until interrogated with an external tag reader, and then it briefly transmits a unique identification number to a receiver. Studies examining the feasibility and ease of PIT tag use determined that PIT tags have a much greater retention rate than external tags and increase the reliability of re-identifying tagged animals (Balazs 1999; Braun-McNeill et al. 2003; Dutton and McDonald 1994) when tag readers are on hand. Due to the subcutaneous placement of PIT tags, internal complications that result after insertion may occur. To date, surprisingly few studies have examined this aspect of PIT tag use in seaturtles (van Dam and Diez 1999).

The negative effects of PIT tags on wild animals are often difficult to determine. Once seaturtles have been tagged, they are released (usually immediately) back into the environment where they spend most of their lives beyond the reach of researchers. PIT tags are designed to minimize internal complications through the use of a glass protective coating. Although this glass casing serves to both protect the electronic components and prevent tissue irritation (Gibbons and Andrews 2004), there are some reports of visible infection and irritation at the PIT tag injection site despite proper use of disinfecting techniques during insertion in marine turtles (Dutton and McDonald 1994), manatees (Wright et al. 1998), and fish (McKenzie et al. 2006). Along the Atlantic Coast of the U.S., reports of tag-related infection and joint injuries appear in stranding data (New England Aquarium [NEAq], unpubl. data). While there are many advantages to using PIT tags (Balazs 1999; Gibbons and Andrews 2004), recognizing risks and advantages associated with different tagging sites will enhance the quality of studies relying on this marking method. As all seaturtle species are considered imperiled (IUCN listing varies with population), there remains a need to recognize impacts on the health, physiology, and ultimately the survival of PIT-tagged turtles.

The use of PIT tags is usually benign. When tags are placed into muscle, the cutting edge of the applicator needle makes a small circular cut that extends to the depth of the injection. The tags induce encapsulation by fibrous connective tissue, which stabilizes their placement. Encapsulation is most rapid and effective in highly vascular, resilient tissue such as muscle. While the injection causes a small cut in the muscle, it is the responses by fibroblasts and muscle cells to this minor damage that result in largely stable tags. When the tags do not stay where placed, they can be expelled from the body or migrate internally causing inflammation and damage and opening a route to infection. PIT tag movement has been documented in bats (Barnard 1989), young birds (turkey poults; Jackson and Bunger 1993), reptiles and amphibians (Camper and Dixon 1988; Keck 1994), and fish (Baras et al. 2000, Gheorghiu et al. 2010). Baras et al. (2000) found that PIT tags injected into perch migrated ventrally over time. PIT tag movement also has been reported in Hawksbill (*Eretmochelys imbricata*) seaturtles (van Dam and Diez 1999). Not only can the migration of the tag reduce the re-identification accuracy, it may cause injury. The risk of complications can be minimized by the location and method of tag placement (Germano and Williams 1993; Gibbons and Andrews 2004; Jackson and Bunger 1993). In cheloniids, PIT tags usually are inserted via injection using an applicator fitted with a sterile 12-gauge needle. The most common PIT tagging sites used in the U.S. Atlantic and Gulf of Mexico coasts are subcutaneously along the trailing side of the flipper blade (adjacent to the radius and ulna, wrist, and/or metacarpals; Fig. 1) or, for large species (*Dermochelys* and some cheloniids), in anterior shoulder muscles medial to the arm. Additionally, more than 9000 Kemp's Ridley (*Lepidochelys kempii*), Loggerhead (*Caretta caretta*), Green (*Chelonia mydas*) and Hawksbill turtles were released with PIT tags inserted into the ventrally located pectoral muscles (Fontaine et al. 1987). To date,

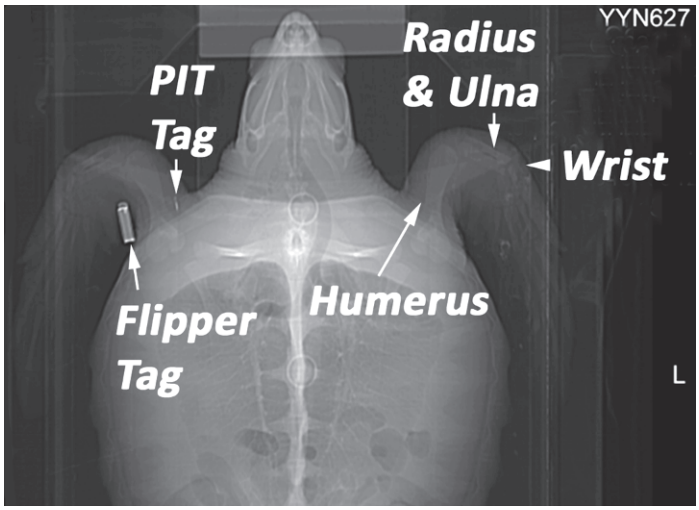

Fig. 1. The overall skeletal anatomy of the marine turtle forelimb and shoulder and several tags are shown in this two-dimensional dorsoventral view CT scan. The forelimb skeletal elements of relevance to this study are labeled. The small PIT tag is in the triceps muscle, an external flipper tag in attached to the trailing edge of the flipper. The light circles at mid-body and the base of the neck, as well as the lighter rectangle at the anterior head, are parts of the scanner's table.

studies of PIT tag placement in seaturtles focus on locations that maximize tag reader reception (Epperly et al. 2008).

In this study, we tested the null hypotheses that PIT tag movement does not differ between species or between two locations in the forelimb: the trailing side of the flipper blade (defined as the proximal manus and antebrachium) and the triceps muscle complex of the upper arm (the brachium). We assess whether PIT tags migrated once placed at the two locations in clinically healthy juvenile Loggerhead and Kemp's Ridley turtles. We report PIT tag movement in both species at the two tagging sites and recommend adoption of the triceps muscle site in seaturtle studies to reduce tag migration and potential complications.

Turtles and Maintenance.—The Loggerhead turtles for this study were wild-caught as hatchlings from marked nests in Clearwater, Florida, USA (emerged from nests 27 August 2000); Kemp's Ridley turtles originated as hatchlings from relocated nests incubated on the beach at Rancho Nuevo, Mexico (emerged 16 August 2000). The turtles were reared in captivity at the National Marine Fisheries Science (NMFS) Sea Turtle Facility in Galveston, Texas, USA. All turtles were juveniles and were similar in size (Loggerhead turtles, N = 21, 31.4–33.6 cm straight carapace length (SCL), mean ± SD = 32.6 ± 0.6 cm; 3.3–4.8 kg, mean = 4.0 ± 0.4 kg; Kemp's Ridley turtles, N = 24, 27.0–30.2 cm SCL, mean = 29.1 ± 0.7 cm; mean = 3.4 ± 2.0 kg). Turtles were held in individual rearing containers in a common raceway tank filled with seawater. Raceways were drained and re-filled with fresh seawater three times/week (described in detail elsewhere, Higgins 2003). Kemp's Ridley turtles were maintained in the Galveston facility prior to and for the duration of the study. Loggerhead turtles were maintained at the Galveston facility for two months, taken to Panama City, Florida and placed under semi-wild conditions for 30 days in communal, large open seawater pens. There they were used for fishing equipment research unrelated to this study, then returned to Galveston. In the Panama City pens, turtles were able to swim more vigorously and interact, potentially challenging the tags and the tag sites beyond what they might experience at the Galveston facility.

PIT Tag Placement and Tag Migration Assessment.—All turtles were tagged in the same forelimb, at both tagging locations, on the same day. Tagging sites were cleaned with 70% isopropyl alcohol and povidone iodine swabs, and then one PIT Tag (Model TX1406L, Destron-Fearing, 12.50 mm L x 2.07 mm diam) was inserted at each site via a pre-loaded sterile 12-gauge needle. One tag was placed deep to the dorsal skin adjacent to the fifth metacarpal and carpal bones on the caudal (postaxial) side of the flipper (Fig. 2a), while the other was placed within the cranial part of the triceps muscle complex in the upper arm on the same side of the turtle (Figs. 2b). Each tag site was sealed with a drop of surgical cement (VetBond™ ; 3M™ , St. Paul, Minnesota) to minimize the chances of tag loss and infection.

Initial MRI scans of the entire flipper were taken shortly after insertion (1 April 2002 for Kemp's Ridley turtles; 2 April 2002 for Loggerheads). Second MRI scans were taken at 104–106

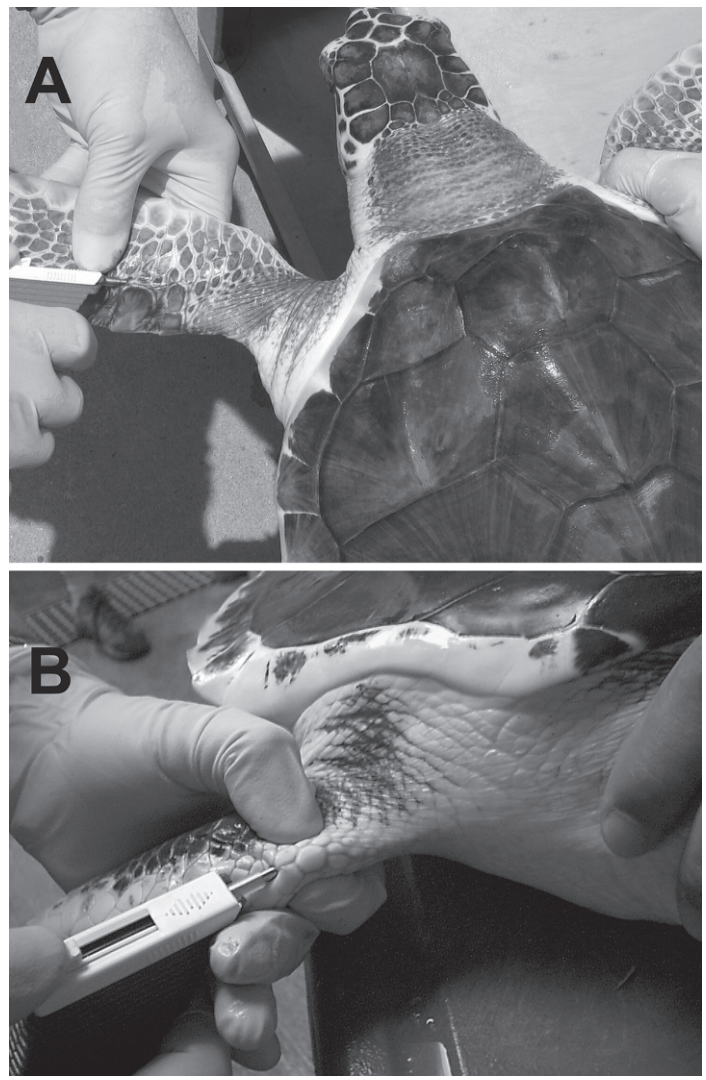

Fig. 2. (A) PIT tag insertion into trailing edge of flipper blade parallel to the fifth metacarpal and postaxial carpal bones. (B) PIT tag insertion in triceps muscle complex of the upper arm showing the method of manipulating the muscle and allowing the tagger to palpate the insertion of the tagging needle and tag.

days (16 July 2002 for Loggerheads, and 17 July 2002 for Kemp's Ridleys). Each turtle was anesthetized (0.15 mg/kg meditomidine, 5 mg/kg ketamine) administered IV in a cervical sinus (external jugular vein). Turtles were placed in ventral recumbency with the flipper positioned alongside the body in a standardized position, flippers were flexed so they rested along the lateral carapace with the blade's long-axis aligned with the scanner table axis, and flippers were held in place with Vet Wrap™ Bandaging Tape (3M™, St. Paul, Minnesota). Scans were taken in dorsoventral and axial planes so that the three-dimensional positions of the skin, muscles, tag, and bones could be visualized. After the scans, the anesthesia was reversed with 0.75 mg atipamezole administered IV in the external jugular vein.

TABLE 1. The fate of PIT tags in (A) Loggerhead (N = 21) and (B) Kemp's Ridley (N = 24) seaturtles over 104–106 days, showing the contingency tables for each species.

A. Loggerhead		Flipper blade		
		No movement	Movement	Row totals
Triceps muscle	No movement	11	7	18
	Movement	1	2	3
	Column totals	12	9	21
B. Kemp's Ridley		**Flipper blade**		
		No movement	Movement	Row totals
Triceps muscle	No movement	15	9	24
	Movement	0	0	0
	Column totals	15	9	24

The metal in each PIT tag produced a signal-void of characteristic size and shape. The maximum size of each signal-void (hereafter referred to as the PIT tag) was used to determine tag position. Each scan was evaluated to determine the distance of the PIT tag's longest axis end to nearest joint and/or bone (Fig. 3); the structure used as the landmark varied among individuals, but was consistent within an individual. Distances were measured using eFilm Lite v.2.1 (Merge Technologies Inc. 2005) and compared. Humerus length (from midpoint of the head to distal-most point, the radial facet) was also measured and compared between first and second scans to account for growth during the study. PIT tags were categorized as having moved or not moved. If the distance of the PIT tag void to the nearest joint and/or bone was greater than the increase in humerus length ± resolution error (defined as > 0.2 cm), the tag was considered to have moved. If the distance was not greater, the tag was assumed to have not moved. In the absence of three-dimensional reconstructions and scan intervals greater than the one we used, we could not measure actual paths traveled for each tag.

Statistical Analysis.—A 2 x 2 contingency table and McNemar's test for significance of changes, adjusted with Williams' correction, was used to assess whether PIT tag movement in one location was independent of movement in the second location. SAS v.9.2 was used to generate all statistics (SAS Institute, Inc. 2009).

Results.—In Loggerheads, three of the 21 tags (14%) placed in the triceps muscle complex migrated, while 9 tags (43%) placed in flipper blades displayed movement (Table 1). However, in the

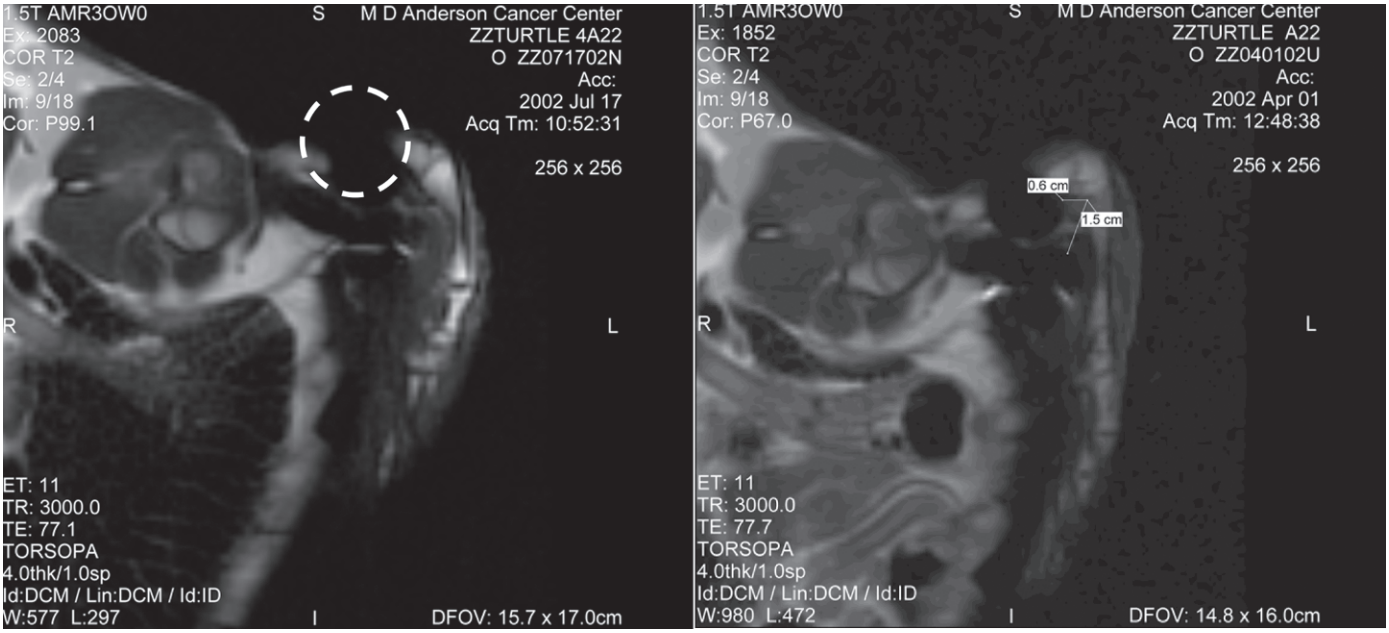

FIG. 3. (A) A dorsoventral plane MRI scan of the left turtle flipper and shoulder. The white dashed circle indicates a signal-void produced from PIT tag in the triceps during the MRI scans. The bone and fat are light colored, muscle is gray, and air in the lung is black. (B) MRI scan of a left turtle flipper with measurements taken from the long axes of the two signal-voids to the nearest joint. Humerus length (from midpoint of the head to the distal-most point, the radial facet) was also measured and compared between first and second scans to account for growth during the study.

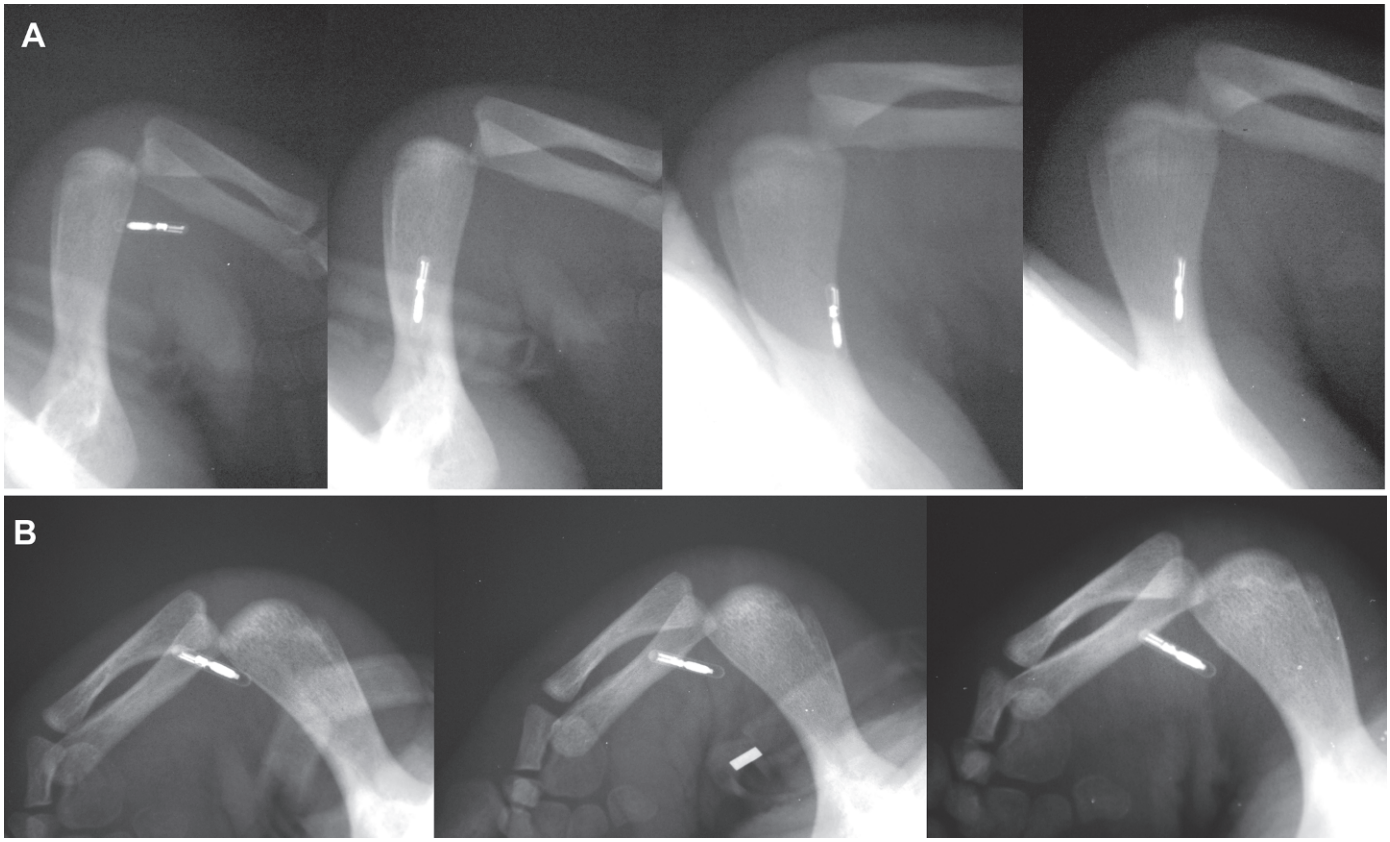

Fig. 4. (A). Migration of a PIT Tag in the right flipper of a cold-stunned Kemp's Ridley (ID No. 99-831). The top four images span 253 days. The greatest proximal movement occurred between the initial radiograph (28 days after the tag was inserted) and the second, a period of 58 days. Subsequent movement was more circumferential from the second to last images (from 58 days to 168 days to 253 days). While the tag migration was quite obvious, the site did not become infected, most likely due to several courses of systemic antibiotics administered to treat other health issues. (B) Botton row: radiographs taken of a second Kemp's Ridley (ID No. 99-842), also show PIT tag migration, however with less drastic movement than in 4A. While the migration of the tag appears shorter in distance, the impact to the animal's health was more significant. The PIT tag was initially placed in the anterior dorsal part of the left front flipper blade on 21 November 1999. The radiographs (left to right) were after two days, 73 days, and 89 days. The tag site became severely infected requiring surgical removal of the tag. The animal was treated topically at the wound site and with systemic antibiotics for several months following tag removal.

majority of cases (62%), both of an animal's tags performed similarly. We rejected the hypothesis that movement at the two sites was the same in Loggerheads ($S_{adj} = 4.2353$, $p < 0.05$). The maximum net tag migration that we could measure, in Loggerheads, was 5.2 cm in the flipper blade and 1.3 cm in the triceps muscle complex. In Kemp's Ridley turtles, none of the 24 tags placed in the triceps muscle complex migrated, whereas 9 of the tags (38%) placed in the flipper blade had moved (Table 1); the maximum net distance moved in the flipper blade of Kemp's Ridleys was 2.2 cm. The marginal proportions (e.g., the cases where both tags in a turtle did not have the same result) were identical (38%) in the two species (Table 1). In no case was infection noted.

Discussion.—We determined that the forelimb tagging locations used in two species of marine turtles differ in their tendency for PIT tags to migrate. Tag migration is an important factor to consider in minimizing risks to the turtles. Movement occurred more often in PIT tags that were inserted subcutaneously in the flipper blade, suggesting that this location can be problematic. The tag stability at one site was not related to tag performance at the other site. Our results are consistent with the tag loss results that vanDam and Diez (1999) found when tags were placed subcutaneously in Hawksbill turtles. Despite the short time frame of this project and the lack of three-dimensional reconstructions, we were able to detect migration by PIT tags in the subcutaneous hypodermal layer, which is rich in collagenous and elastic connective tissues. This site, adjacent to the ulnare, pisiform (carpals), and fifth digit metacarpal, has relatively thick, keratinous skin overlying a connective tissue network with little or no muscle tissue. The muscles of the manus are very reduced in this part of the flipper and may be missed entirely during PIT tag application. In contrast, PIT tags inserted into the triceps muscle complex with the muscle pinched outward (cranially or dorsally) during tag placement (Fig. 2b) clearly are embedded in muscle tissue. Muscle, a metabolically more active tissue, is more likely to encapsulate the tag quickly. While PIT tags may become encapsulated and stabilized in connective tissue, they appear to be more stable when placed in muscle. We presume that the tags placed in the shoulder muscles of large turtles as well as those placed in the hind flipper muscle (Balazs, unpubl. data), a tagging site used in some Pacific studies, are probably stable, however, direct comparisons with the hind flipper site were beyond the scope of this study.

The possibility exists that movement of the tag may occur well beyond the time frame of this study (104–106 days). It is possible

that tag movement or loss may be greater in wild animals than those in captivity (Thomas 2006) associated with their potential exposure to more varied physical stressors, although we did not observe such in Loggerheads that were allowed 30 days of swimming in open pens (compared with Kemp's Ridleys that were maintained in tanks). PIT tag movement may also increase with the size of an animal (Gibbons and Andrews 2004), indicating that the tag movement measured in this study may be less than what might occur in larger juvenile or adult turtles.

In some cases, movement of a PIT tag may be detrimental to seaturtle health and survival. Several cases of PIT tag migration in the flippers of cold-stunned Kemp's Ridley turtles were documented by the NEAq while the animals were undergoing rehabilitation. In November 1999, more than 277 seaturtles stranded along the shores of Cape Cod Bay (Massachusetts, USA) during a large cold-stunning event. A total of 156 were recovered alive (Still et al. 2002) and transported to the NEAq for rehabilitation. Due to the overwhelming volume of cases admitted into the NEAq seaturtle clinic, turtles were rapidly transported to secondary facilities for rehabilitation. Many turtles were PIT tagged in the dorsal and cranial flipper blades prior to transport (N = 103 Kemp's Ridleys), while others were tagged prior to release. Tag migration was noted via radiographs of several turtles (Fig. 4). At least eight of those 103 turtles developed localized infection associated with the PIT tag, and surgical removal of the tag was required. It is likely that this number under-represents the true number of infections, due to the difficulty in following the clinical outcome of large numbers of turtles after relocation to multiple institutions. At least one of these turtles developed significant osteomyelitis of the humerus, radium, and ulna. While osteomyelitis and joint mobility issues are common in cold-stunned turtles (Wyneken et al. 2006), the proximity of the PIT tag to the sites of infections suggested further assessment of PIT tagging location was warranted. Infections of the skeleton and primary locomotor structures are likely to decrease survival probability.

Because our study tested the tendency of PIT tags to migrate in clinically normal turtles (both Kemp's Ridley and Loggerhead turtles) we were able to confirm that tags placed in the flipper blade may migrate even in the absence of infection or cold stress. Similarly, tag migration was observed in penguins both in the presence and absence of microbial growth around the tag and infection (Clarke and Kerry 1998).

It is likely that when physiologically stressed, such as by hypothermia, immune function decreases (reviewed by Jacobson 2007). Thus cold-stunned or otherwise stressed turtles may be at increased risk of infection in the PIT tag site (Baras et al. 2000). Infection and irritation have been reported in PIT tagged leatherback turtles as well (Dutton and McDonald 1994).

Studies of PIT tag placement in the skin of manatees (*Trichechus manatus latirostris*) showed that when the skin is thick, a plug of skin can be driven internally in front of the tag during the injection, increasing the risk of infection. This may, in turn, increase tag movement or rejection (Lambooij et al. 1995; Wright et al.1998).

PIT tags can also be expelled from animals, depending on where the tag is placed (Elbin and Burger 1994; Fontaine et al. 1987; Gibbons and Andrews 2004). Zimmerman and Welsh (2008) studied the placement of PIT tags in American Eels (*Anguilla rostrata*) and found that tag retention varied according to tag location, with the highest retention rates in tags placed into musculature. PIT tags placed in penguins and monitored over several years showed that tag movement can be substantial (> 5 cm; Clarke and Kerry 1998). We observed less movement in PIT tags placed into seaturtle triceps muscle than the trailing aspect of the flipper blade, thus its use may minimize the potential for tag migration, infection and loss. Risk of PIT tag movement and/or infection caused by PIT tags in seaturtles can be mitigated by combining standard skin cleaning with an antiseptic solution prior to application, the use of a sterile applicator and tag, and placement of the tag in a stable location where the keratinous skin layer is thin and the tag is most likely to be placed in muscle. In addition, the accuracy of seaturtle mark-recapture studies will increase because once placed in the triceps muscle, the tag is less likely to move; it remains within readable distance because of the location and size of the muscle, and should increase recognized recaptures.

There may be other advantages to placing the PIT tags into the triceps muscle over the flipper blade. The triceps muscle complex provides some soft tissue protection and so may decrease the likelihood of a tag failure due to shattering of the glass casing. When the glass encasing a PIT tag's electronics is broken, the transponder fails (Camper and Dixon 1988; Lambooij et al. 1995) and tags can migrate, breaking through skin (Germano and Williams 1993). Camper and Dixon (1988) report tag breakage and malfunction due to aggressive encounters between lizards. While we found no incidence of PIT tag failure in seaturtle flipper blades during this study, lack of "padding" in the flipper blade does little to minimize the risk of tag breakage.

While PIT tag location, consistency, and reliability are important aspects of any mark-recapture study, there has been relatively limited consideration of the efficacy of tag placement in seaturtles except for turtle size (Fontaine et al. 1987) and accommodating the limits of tag readers (Epperly et al. 2008). Consideration of tag migration risk in seaturtles is examined for the first time here.

The use of PIT tags is an integral part of much herpetological research. PIT tag use is clearly essential in understanding vertebrate populations worldwide and has greatly increased the reliability and ease of re-identification of individuals. PIT tag use is bolstering our knowledge of seaturtle populations, at sea and on land. Their use in seaturtles has increased recapture rates, which has improved understanding of turtle movements, growth rates, habitat use, nesting success, internesting intervals, migration and numerous other life-history attributes. In addition, the use of PIT tags has eliminated the need to mark turtles using tattooing, drilling or carapace scarring (Affronte and Scaravelli 2002; Hendrickson and Henrickson 1981). Our results support avoiding the flipper blade and applying tags in the triceps to minimize risks of compromising the well-being of the tagged animal.

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vice Endangered Species Act Section 10a(1)a Scientific Research Permit #TE-676379-4 and complied with all institutional animal care guidelines. The Florida Atlantic University (FAU) IACUC determined that the study is in compliance with IACUC guidelines; administratively it does not receive an approval number because FAU personnel worked with the data files alone, not the animals. The methods used were consistent with those the IACUC would approve had the animal part of the study been done by FAU personnel. At the time, the NMFS did not issue IACUC approvals, but always complied with USFWS and State of Florida requirements for holding seaturtles. J. Flanagan, DVM ensured that best practices were followed. All authors participated in one or more aspects of this study and edited the manuscript.

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