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Guest Editorial: Research Needed to Develop an Improved Life-long Living Tag Applicable to Carapace Scutes of Emergent Hatchling Kemp's Ridley Sea Turtles

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Among the many contributions made by John R. and Lupe P. Hendrickson were their pioneering development and testing of living tag autografts as life-long marks for sea turtles (Hendrickson & Hendrickson 1980, 1981a,b, 1983, 1984, 1986; Balazs 1999; Kishinami 2003; Owens 2003; Mrosovsky & Godfrey 2003; Bell *et al*. 2005; Mrosovsky 2007). Their vision for the living tag was mass-tagging emergent hatchlings to test the hypothesis of natal beach imprinting and to provide data on many other aspects of sea turtle behavior and population dynamics. For Kemp's ridley (*Lepidochelys kempii*), mass-tagging of emergent hatchlings (both sexes) with life-long living tags would be comparable to the mass-tagging them with "archival" tags, which was recommended by Eckert *et al*. (1994) as a means of determining hatchling-to-adult survival rate, average juvenile-to-adult survival rate, juvenile growth rates, behavior (habitat selection, movement, and migration patterns), physiology (physical fitness), sex ratios of in situ populations, size frequency distributions of juveniles, and age to maturity (see review by Caillouet *et al*. 2015).

In experiments on hatchlings and juveniles of several sea turtle species, Hendrickson & Hendrickson (1980, 1981a,b, 1983, 1984, 1986; Balazs 1999) excised small samples of plastron and carapace scute tissues from individual turtles and grafted them into the wounds at the opposite locations from which they were excised. The plastron-to-carapace autograft became the most commonly and successfully used living tag (Fontaine *et al*. 1993; Bell *et al*. 2005; Mrosovsky 2007; NMFS SEFSC 2008; Caillouet *et al*. 2015; Shaver & Caillouet 2015). Caillouet *et al*. (2015) suggested that a less invasive, non-surgical, living tag be developed for marking large samples of Kemp's ridley hatchlings to identify their year-class and natal beach origin.

Herein we use Kemp's ridley as our primary example; both the plastron and carapace of its newly emerged hatchlings are dark gray or black (Marquez-M. 1994), demonstrating

that both were pigmented during embryological development. Curiously, the plastron of Kemp's ridleys reared in captivity becomes white within 6-7 months (i.e., it loses its pigmentation), but the carapace remains black or dark gray in 1-year-olds (Marquez-M. 1994). Observations made at the NMFS Galveston Laboratory indicate that the contrast between plastron and carapace color can differ depending on background color and lighting of rearing containers and surroundings, especially through changes in pigmentation of the carapace. Such changes have also been reported in freshwater turtles reared in captivity (Rowe *et al*. 2013, 2014a,b). However, in the wild, the vivid contrast in coloration between plastron and carapace in Kemp's ridley exists at least through the 2-yr oceanic life stage. When free-living (wild) Kemp's ridleys enter the neritic life stage, their carapaces begin to lighten in color but still remain darker than their plastrons through maturity (Marquez-M. 1994). Remarkably, plastron autografts into carapace scutes remain lighter in coloration than the carapace, and they grow larger as the carapace scute increases in size (Fontaine *et al*. 1993). Living tags have been recognized and documented in Kemp's ridleys in the wild (Caillouet *et al*. 2015; Shaver & Caillouet 2015). For Kemp's ridley, the ten costal scutes are the best choices for application of living tags to emergent hatchlings. Marking single costal scutes of emergent hatchlings with the living tag would provide unique identification for 10 year-classes (i.e., cohorts); marking combinations of two costal scutes would provide unique identification for 45 more year-classes (i.e., cohorts) of hatchlings. Thus a total of 55 year-classes could be uniquely marked with living tags, before use of any single or double scute mark would have to be repeated.

In the past, costal and other carapace scutes of head-started (i.e., "yearling") Kemp's ridleys were marked with living tags and the turtles were released into the Gulf of Mexico (Fontaine *et al*. 1993; Caillouet *et al*., 2015), and it is possible that some of these turtles have survived to the present. In any case, plastron-to-carapace living tags on carapace scutes have proven useful in identifying the year-class and nesting beach of origin of Kemp's ridley recaptured or stranded in wild, or found near or on nesting beaches (Caillouet *et al*. 2015; Shaver & Caillouet 2015).

Anticipating development of the living tag, Solomon *et al*. (1986) examined carapace and plastron tissues of juvenile green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles of unspecified sizes. They found that carapace tissue of juveniles was heavily pigmented but plastron tissue was unpigmented, although isolated melanin granules existed within the epidermal and keratinized layers of plastron tissue at the subcellular level. Presence of isolated melanin granules in plastron tissue of juveniles demonstrated that melanin-producing cells (melanocytes) had been present. Melanocytes are the most abundant pigment-producing cells in turtle carapaces (Solomon *et al*. 1986; Alibardi & Thompson 1999; Lindgren *et al*. 2014). Solano (2014) reviewed melanin types, structural models, biological functions, and formation routes in reptiles, etc.

We recommend that experiments be conducted in the laboratory to determine efficacy of known anti-melanogenic agents and treatments in reducing pigmentation in hatchling sea turtle carapace scute melanocytes. The goal of such research would be to develop non-surgical, less invasive methods of creating life-long, easily recognizable, living tags for use in mass-tagging emerging hatchlings to identify their year-class. Objectives include development of an improved living tag that would (1) be applicable to large numbers of hatchlings of single cohorts, (2) be easier and less time-consuming to apply than plastron-to-carapace autografts, (3) grow in size with growth of the scutes as do plastron-to-carapace autografts, and (3) be permanent, and no less recognizable than plastron-to-carapace autografts. There exists an extensive literature on anti-melanogenic effects of various agents and treatments on melanocytes (e.g., Schwartzkopf *et al*. 1994; Van Den Boorn *et al*. 2011; Baek *et al*. 2014; Obagi & Kenkel 2014). Many such studies have been conducted on freshwater turtles (e.g., Alibardi & Thompson 1999; Hou 1999; Bragulla & Homberger 2009; Hou & Hou 2010).

General requirements for sea turtle tags and tagging are described by Witzell (1998), Balazs (1999), Eckert & Beggs (2006), NMFS SEFSC (2008), Plummer & Ferner (2012), Dutton & Stewart (2013), and the Cooperative Marine Turtle Tagging Program <http://accstr.ufl.edu/resources/tagging-program-cmttp>. We suggest the following standards for non-surgical, living tag marking methods applied to carapace scutes of emergent sea turtle hatchlings: (1) no more invasive, painful, or harmful to hatchlings than plastron-to-carapace autografting, injection of coded-wire tags (CWT), injection of passive integrated transducer (PIT) tags, or sampling for genetic tagging or chemical analyses (Fontaine *et al*. 1993; Fitzsimmons *et al*. 1999; Lukacs & Burnham 2005; Eckert & Beggs 2006; Reich *et al*. 2007; NMFS SEFSC 2008; Plummer & Ferner 2012; Dutton & Stewart 2013), and (2) applicable to marking one or more carapace scutes (Pritchard & Mortimer 1999) to increase the number of unique codes used to identify cohorts.

We suggest that initial studies be conducted in the laboratory on red-eared sliders (Trachemys scripta elegans, an invasive species) reared for 1-2 yrs in captivity, using the following experimental approach:
(1) Apply anti-melanogenic agents and treatments to cell cultures of carapace scute melanocytes from emergent hatchlings (Hou 1999; Hou & Hou 2010).
(2) Agents and treatments that produce the most promising results on carapace scute melanocytes in cell culture should be tested by application to carapace scutes of living emergent hatchlings.
Experiments on carapace scutes of emergent hatchlings should include anti-melanogenic agents applied topically and by injection, Q-switched laser treatment, liquid nitrogen branding, etc. For treatments that may cause pain, topical or injected anesthetics should be applied. For topical application of an anti-melanogenic agent, it may be necessary to mix the agent with water-resistant adhesive so that the agent remains in contact with the

scute long enough to be permanently effective, but the adhesive should not inhibit or prevent scute growth. If any of these approaches show promise, they should then be repeated experimentally on Kemp's ridley cell cultures; those shown to be safe and effective should then be applied to carapace scutes of emergent hatchlings reared in captivity long enough to evaluate results. If proven safe and effective for marking emergent Kemp's ridley hatchlings, these approaches could then be applied to mass tagging emergent hatchlings. The short generation time and limited geographic distribution of Kemp's ridley are advantageous to developing and testing this life-long tag. Further testing in the field will be necessary, by mass-tagging emergent hatchlings of several consecutive year-classes and assessing tag returns. All testing on Kemp's ridleys will require various permits.

Compared to other external and internal tags, as well as DNA, used to identify sea turtle cohorts or individuals, detection and interpretation (decoding) of living tags requires no special equipment or additional tissue sampling upon recapture. Visual identification by trained observers has proven sufficient to detect and decode living tags (Bell *et al*. 2005; Caillouet *et al*. 2015; Shaver & Caillouet 2015). However, observers must be aware of living-tagging programs and carapace scute nomenclature (NMFS SEFSC 2008). Thus, novice observers probably would not recognize or report living tags, but this also applies to internal tags and DNA. As for all other sea turtle tagging methods, detection of living tags will depend upon diligence in examining all encountered Kemp's ridley for living tags (Caillouet *et al*. 2015).

Lack of familiarity with living-tagging programs or mistaking living tags for marks made by injuries can prevent reporting of living tags or cause erroneous reporting of injury marks as living tags (Balazs 1999; Caillouet *et al*. 2015; Shaver & Caillouet 2015). The number of year-classes that can be uniquely living-tagged can be increased by marking combinations of two costal scutes. However, it may not be necessary to mass-tag many consecutive year-classes of emergent hatchlings with living tags to meet objectives, but like any other tagging methods, it will take decades to collect returns. The Cooperative Marine Tagging Program can provide for archival of information on chosen carapace scute locations of living tags, numbers of emergent hatchling Kemp's ridleys tagged by year-class, and documented tag returns.
Obviously, development of improved methods of creating living tags on emergent hatchling Kemp's ridley carapaces is long-term, but such tags would be very useful. The most practical use of improved living tags applied to emergent hatchlings would be to identify year-classes and nesting beach origins of adults, particularly adult females on nesting beaches and adult males near nesting beaches.

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