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Diurnal nesting female hawksbill turtle at Curieuse Island National Park, Seychelles
(see pages 6-11). Photo credit: April J. Burt

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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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Guest Editorial: TEDs, Delayed Mortality, and Strandings of Sea Turtles That Otherwise Appear Normal and Healthy

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When I searched the literature for my earlier editorial on delayed mortality in sea turtles (Caillouet 2012), I failed to discover Anonymous (2006), which is relevant to the topic of strandings of sea turtles that otherwise appear normal and healthy. Anonymous (2006) suggested that a significant proportion of sea turtles that exit shrimp trawls via TEDs have sustained injuries or suffered irreversible shock in the trawls before being shunted out. Anonymous (2006) referred to a controlled experiment conducted by Schwartz (2001) that demonstrated that “even when sea turtles survived forced submergence and seemed to recover from oxygen deprivation, they died hours later.” Anonymous (2006) stated further that “A turtle struggling to get to the surface to breathe may not find the TED exit until it succumbs to anoxia and becomes passive and physiologically damaged. That damage would be multiplied if an individual was

caught and shunted out multiple times, as seems likely when large numbers of shrimp boats are trawling close together in the same area.” I apologize for my oversight.

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Plastic Straw Found Inside the Nostril of an Olive Ridley Sea Turtle

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The increasing quantity of plastic debris in the oceans pose a growing threat to marine life (Derraik 2002, Cózar *et al.* 2014). Large amounts of plastic debris are often reported in the gastrointestinal tracts of necropsied sea turtles (Plotkin *et al.* 1993, Lazar & Gračan 2011, Schuyler *et al.* 2014), but only rarely is it possible to report on the potential impacts of plastic debris on living turtles. Here, we describe an encounter with a male olive ridley turtle *Lepidochelys olivacea* that had a plastic straw deeply embedded into its nostril.

On 10 August 2015, we were conducting boat surveys in search of olive ridley sea turtles in the waters between Playas Del Coco and Playa Nancite on the Pacific coast of Costa Rica. At N 10°40'37.9" W 085°42'32.6", we encountered a pair of mating olive ridley turtles with an additional male turtle close-by. The non-mating male was captured by hand and brought on the boat for tagging and tissue sampling. Upon inspecting the animal at close range an encrusted cylindrical object was discovered in the sea turtle's left nostril. Initially (Figure 1a), we presumed that the object was the tube from an epibiotic tube worm and attempted to collect the specimen for cataloguing. Using a pair of pliers from a Swiss army knife we gently pulled on the object until the object was protruding from the nostril by approximately 1 cm (Figure 1b). Surprised at the length of the object and still not knowing exactly what it was, we trimmed off the protruding end for further investigation. Upon closer inspection, it became clear that the object was a rigid plastic straw. Bringing the turtle in for veterinary treatment was not an option as our permits allowed only for the turtle to be held on the boat for the regular sampling procedure (tissue sampling and tagging). Furthermore, we were 1-2 hours from port and had no assurance that appropriate treatment would be available even if the turtle was taken to the nearest veterinary clinic or hospital. Thus, we decided to remove the straw *in situ*. After a few short pulls, the entire straw, which measured a total 10 cm in length, was extracted (Figure 1c). By this point, the turtle was bleeding out of its nose. We applied Betadine® solution to the nostril and in less than a minute the bleeding stopped. With the turtle breathing clearly and appearing healthy in all other respects, it was returned to the ocean and swam away.

We do not know conclusively how long the straw had remained in the sea turtle's nostril or how it came to be there in the first place but we can provide some basic conjectures. Judging by the stained coloration of the straw and its general state of degradation (Figure 1c), we predict the straw must have been in the turtle for at least a few weeks if not longer. Furthermore, the effort required to remove the straw suggests that scar tissue had begun to form around the base of the straw. As for how the straw found its way into the sea turtles nostril, we think it is highly unlikely that the straw would have been driven into the sea turtles nose from the outside (*e.g.*, if the turtle impaled its nostril on a straw that may have been sticking out of the seabed); the straw was too deeply embedded into the nostril. Instead, we believe that the straw was initially ingested

orally but was later regurgitated; however, when this happened the straw did not pass out the mouth but passed through the nasal cavity. In support of this argument, the nasal cavity in sea turtles is linked directly to the buccal cavity through a long nasopharyngeal duct (Wyneken 2001). Furthermore, the curve of the straw after removal (Figure 1c) matches the anatomical form of the nasopharyngeal duct (Wyneken, 2001).

The surprising discovery of a plastic straw lodged into the nose of an olive ridley turtle is a single example of the multitude of effects that plastic debris can have on marine life. While the impact of the straw was not immediately fatal, it evidently blocked the nasal

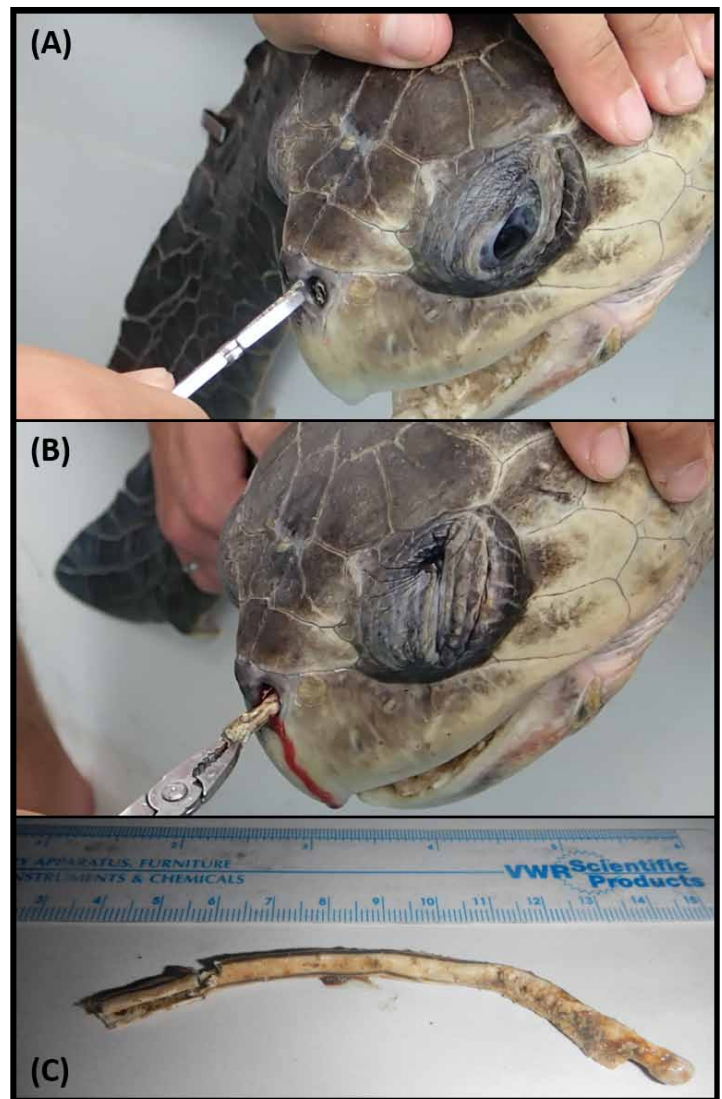


Figure 1. (A) Plastic straw in the left nostril of an olive ridley sea turtle. (B) Removal of the straw. (C) The straw next to a ruler for scale.

passage of the turtle and detrimentally affected the turtle's capacity to breathe. In turn, this could markedly lower this animal's reductive fitness by reducing its ability to feed or even mate. Considering the plastic is found ingested by all sea turtles species (Schuyler *et al.* 2014) as well as many fish (Boerger *et al.* 2010) and seabirds (Tanaka *et al.* 2013), we propose that strategies for reducing plastic pollution in the oceans should play a central role in broad-scale conservation management strategies, not just for sea turtles, but all marine life.

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Curieuse National Park, Seychelles: Critical Management Needs for Protection of an Important Nesting Habitat

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The Republic of Seychelles hosts the largest national population of nesting hawksbills (*Eretmochelys imbricata*) in the Western Indian Ocean, a species listed as Critically Endangered by IUCN (Meylan & Donnelly 1999; Mortimer & Donnelly 2008). In Seychelles most hawksbills nest on beaches of the Inner Islands, the Amirantes Group, and Platte and Coëtivy islands (Mortimer 1984), which are all located within 400 km of the human population centres on the granitic islands of Mahé, Praslin and La Digue where 99% of people live (Fig. 1). In recent history (Mortimer 1984) hawksbills nested on most beaches of the granitic islands, but today the largest populations are restricted to a small number of protected islands. In contrast, green turtles (*Chelonia mydas*), which are listed as Endangered by the IUCN (Seminoff 2004), nest primarily on the remote outer islands and have become rare in the inner islands.

Turtle population declines are attributable to: centuries of international trade in hawksbill carapace and plastron scutes ('tortoiseshell'), a practice which intensified from the mid-1960s until 1992 (Meylan & Donnelly 1999; Mortimer 1984); and exploitation of green turtle calipee, an edible gelatinous substance found under the plastron, formerly used in Europe to make "turtle

soup," especially at the turn of the 20th century (Hornell 1927). Unlike most populations of hawksbills that nest at night, Seychelles hawksbills nest in the daytime, which makes them particularly vulnerable to capture (Mortimer & Bresson 1999) and to disturbance.

Prior to 1994 when Seychelles passed the "Wild Animals (Turtles) Protection Regulations" that banned the killing of turtles throughout the country, sea turtles were only protected within the nature reserves at Curieuse and Ste. Anne National Parks, at Aride and Cousin Island Special Reserves, and at Aldabra Atoll (Mortimer 1984). In 1979 Curieuse Island was designated a Marine National Park, and Park Rangers were stationed on the island to protect turtles and other resources. With a land area of 2.86 km², Curieuse is the fifth largest of the inner granitic islands and is a popular destination for tourists. It has a rocky coastline interspersed with seven nesting beaches with a total length of approximately 2,060 m. At Curieuse, situated 1.2 km north of the second largest human population centre on Praslin Island (Fig. 1), turtles have been subjected to poaching by local people, though to a lesser degree than at other islands; and tourists also cause inadvertent disturbance to nesting turtles. Even though turtle protection has not been perfect (Mortimer 1984, 2004),

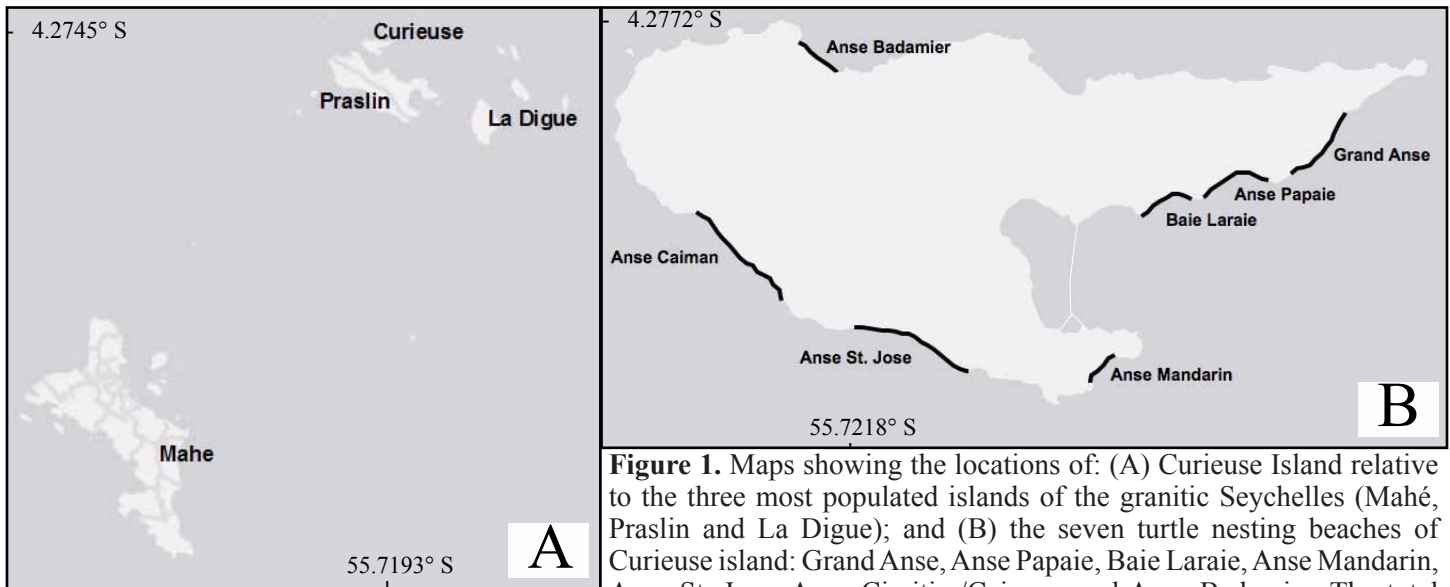


Figure 1. Maps showing the locations of: (A) Curieuse Island relative to the three most populated islands of the granitic Seychelles (Mahé, Praslin and La Digue); and (B) the seven turtle nesting beaches of Curieuse island: Grand Anse, Anse Papaie, Baie Laraie, Anse Mandarin, Anse St. Jose, Anse Cimitier/Caiman, and Anse Badamier. The total area depicted in these maps represents <0.5% of the total Exclusive Economic Zone of Seychelles (1.4 million km²).

Curieuse has managed to retain one of the most important hawksbill nesting populations in the granitic islands of Seychelles and it also hosts a small number of nesting green turtles.

The hawksbill nesting season in Seychelles spans September to April, with a peak during October to January at both the Inner Islands (Mortimer & Bresson 1999) and the Amirantes group (Mortimer *et al.* 2011a). In contrast, seasonal patterns of green turtle nesting vary not only between sites in the western Indian Ocean region (Dalleau *et al.* 2012) but to some degree also from one year to the next (Mortimer *et al.* 2011b; Mortimer 2012). During the three decades (30 nesting seasons) from 1979-1980 to 2009-2010, the Seychellois Park Rangers recorded turtle track counts and tagged turtles at Curieuse while carrying out their anti-poaching patrols during the peak months of the hawksbill nesting season. In most years surveys at Curieuse were incomplete and provided only minimum estimates of numbers of egg clutches laid, except for hawksbills during 1981-1983 and 2001-2003 (Mortimer 2004; Rulie 2002), and more recently for both hawksbills and green turtles since

2010 when Seychelles National Parks Authority (SNPA) joined forces with Global Vision International (GVI), a conservation and community development organization that now collects data on behalf of SNPA.

In this study at Curieuse, we assessed the nesting of hawksbills during the 44 months between September 2010 and April 2014, and of green turtles during the 33 months from September 2012 to May 2015, using the following parameters: a) spatial distribution of nesting activity on seven beaches; b) seasonal distribution of green turtle nesting; c) numbers of egg clutches laid by each species; and d) estimated numbers of females nesting annually. We also recommend long term conservation management strategies for nesting turtles and their habitats at Curieuse National Park.

Data for this study were collected from September 2010 through May 2015 by a total of 35 GVI staff and over 350 volunteers under the guidance of SNPA. Surveys were conducted most frequently and consistently during the hawksbill nesting season, September through April, when Anse Cimitier/Caiman was surveyed 1-2

Beach	m	% of total	Turtle tracks per season				Egg clutches laid per season			
			mean % of total	mean (SD)	mean density per 100m	mean (SD)	mean % of total	mean density per 100m	mean % nesting success per season (SD)	
Grand Anse	320	15.5	276.8 (81.7)	68.4	86.5 (25.5)	144.5 (50.3)	74.9	45.2 (15.7)	51.8 (0.1)	
Anse Papaie	160	7.8	45.5 (10.9)	11.3	28.4 (6.8)	18.8 (8.8)	9.7	11.7 (5.5)	40.2 (0.1)	
Baie Laraie	140	6.8	3.8 (1.7)	0.9	2.7 (1.2)	1.5 (1.3)	0.8	1.1 (0.9)	35.4 (0.3)	
Anse Mandarin*	100	4.9	13.5	3.3	13.5	6.5	3.4	6.5	47.8	
Anse St Jose	780	37.9	20.0 (10.5)	5.0	2.6 (1.3)	7.8 (2.2)	4.0	1.0 (0.3)	48.6 (0.3)	
Anse Caiman	300	14.5	29.5 (11.8)	7.3	9.8 (3.9)	7.5 (6.0)	3.9	2.5 (2.0)	24.6 (0.2)	
Anse Badamier*	260	12.6	15.5	3.8	6.0	6.5	3.3	2.5	46.7	
Entire Island	2,060	100.0	404.5 (97.6)	100.0	18.9 (4.7)	193 (67.8)	100.0	9.1 (3.3)	42.2 (0.1)	

Table 1. Seasonal nesting activity recorded at each of the beaches of Curieuse Island over a four-year period. For each beach the following are indicated: beach length (m); mean estimated numbers and density of turtle tracks produced and egg clutches laid; and mean percent nesting success (defined as percent of nesting emergences that resulted in egg clutches laid).

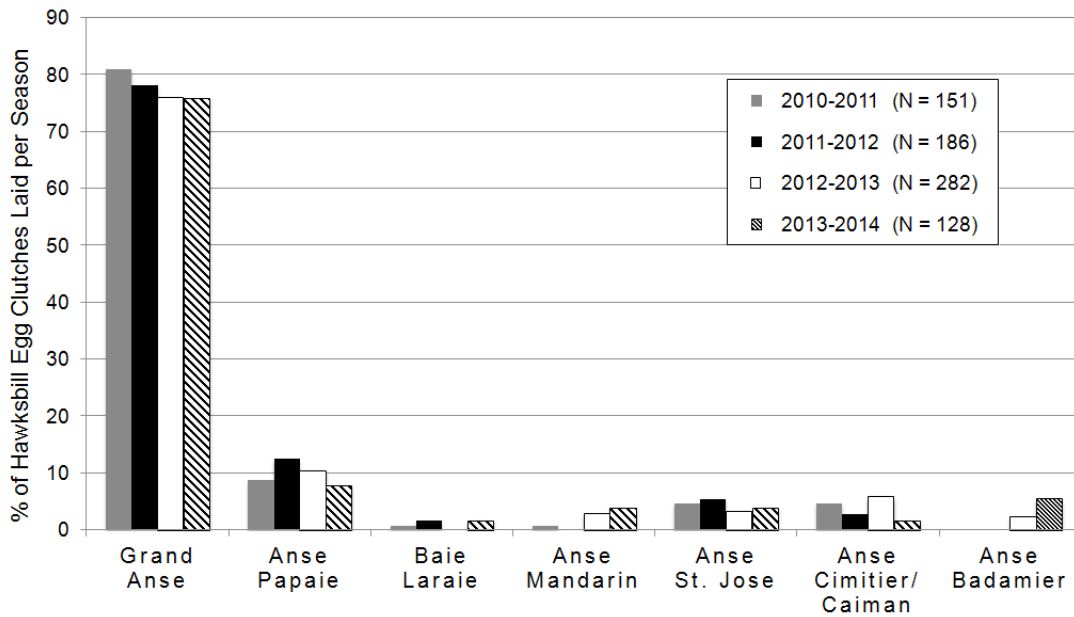


Figure 2. Percentages of total hawksbill turtle egg clutches laid each season at each of seven beaches. Legend indicates total numbers of egg clutches laid during each of the four seasons.

times per week, Grand Anse and Anse Papaie 3-5 times per week, and Baie Laraie and Anse St. Jose 7 times a week. Anse Mandarin and Anse Badamier were not surveyed in 2011 and 2012, but were surveyed 1-2 times per week during 2013 through mid-2015. With this survey schedule we are confident in our assessment of hawksbill nesting activity. During the non-hawksbill season (May through August), beaches were not surveyed regularly before September 2012. But, from September 2012 through mid-2015, all beaches were surveyed at least 1-2 times per week; and because most green turtle body pits remain visible for at least a week, we are confident this survey regime adequately documented all green turtle nesting activity between September 2012 and May 2015 (Fig. 3).

Each turtle track observed was recorded and classified according to species and also whether or not eggs were laid. Those data enabled an assessment of the spatial distribution of nesting emergences and egg laying amongst the seven beaches and an estimate of the total numbers of egg clutches laid by each species each season. Prior to 2010 and during the 2013-2014 season, flipper tags were applied to all nesting hawksbills encountered at Curieuse. New tags were not applied during the 2010-2011, 2011-2012, and 2012-2013 seasons; but throughout the study, tag numbers were recorded whenever a previously tagged turtle was intercepted. No nesting green turtles were intercepted.

The spatial distribution of hawksbill egg clutches laid each season at the seven beaches is presented in Fig. 2. Total activity varied between seasons, but spatial distribution was relatively

consistent. Grand Anse hosted 75-81% of egg clutches laid and Anse Papaie, 8-12%. Table 1 compares the beaches in terms of relative density of hawksbill tracks and egg clutches laid per season, and nesting success (defined as percent of emergences that resulted in egg clutches laid). Nesting density was highest at Grand Anse and Anse Papaie, which together comprise only 23% of the total beach length, but hosted 83-93% of each season's total egg clutches (Fig. 2, Table 1). Nesting success was also highest at Grand Anse, at 52%.

Green turtle nesting (during September 2012 to May 2015) was restricted to only three beaches and distributed as follows: Grand Anse - 63 tracks, 25 clutches; Anse Papaie - 5 tracks, 3 clutches; and Anse Cimitier/Caiman - 1 track, 1 clutch. Together, Grand Anse and Anse Papaie accounted for 98.6% of all green turtle tracks, and 96.6% of the green turtle egg clutches laid.

On average, over the four hawksbill seasons, with each season defined as July to June annually, but with almost all nesting activity during the months of September to April, there was an average of 381 hawksbill nesting emergences (sd = 97.0; range = 312-522) and 187 egg clutches laid (sd = 67.8; range = 128-282) annually (Table 2). During the 33 month-long comprehensive green turtle track study, 69 nesting emergences and 29 egg laying events were recorded (Fig. 3). These data suggest that, although green turtles nest year-round, their nesting season can be defined as April to March annually, with most nesting during the months of June to February. It follows that during the two green turtle seasons for which we have complete surveys (2013-2014 and 2014-2015) an annual average of

	2010-2011	2011-2012	2012-2013	2013-2014	Mean (sd)
Turtle tracks	312	367	522	323	381 (97.0)
Clutches laid	151	186	282	128	186 (67.8)
Mean estimated nesting females (bracketed mean)	43.1 (38-50)	53.1 (47-62)	80.6 (71-94)	36.6 (32-43)	53.4 (46.7-62.3)

Table 2. Current status of the hawksbill nesting population as indicated by total numbers of turtle tracks produced and egg clutches laid during each of four nesting seasons for July 2010 to June 2014. Numbers of nesting females are estimated based on a mean of 3.5 and a bracketed mean of 3-4 egg clutches per female (Mortimer & Bresson 1999; JA Mortimer & R Bresson, unpublished data).

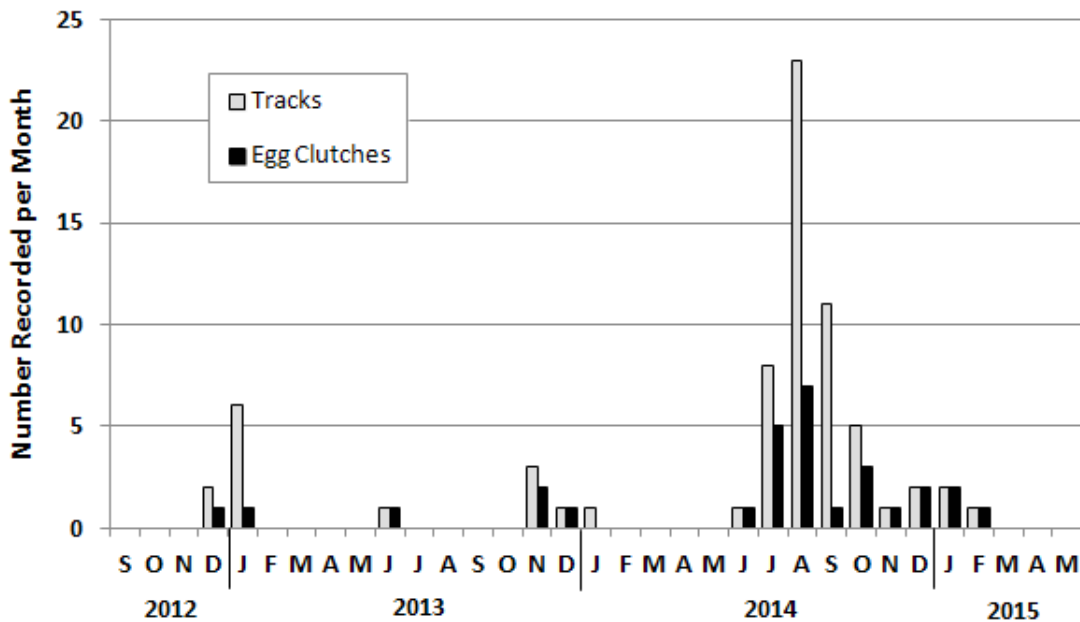


Figure 3. Seasonal distribution of green turtle egg clutches and tracks (nesting emergences) recorded each month at Curieuse during the comprehensively surveyed period from September 2012 to May 2015.

only 30.0 turtle tracks (range = 6-54) and 13.5 egg clutches (range = 4-23) were produced (Fig. 3).

Bracketed estimates of total females nesting per season at Curieuse were calculated from track count data based on the following assumptions: a) that the average female hawksbill lays 3-4 egg clutches in a season, using data from Cousin (Mortimer & Bresson 1999) and Bird (JA Mortimer & R Bresson, unpublished data) islands; and b) that the average green turtle lays 3-5 clutches per season, using data collected at Aldabra Atoll (Mortimer *et al.* 2011b). In this manner we estimated that an average of 53.4 individual female hawksbills (range = 46.7-62.3) (Table 2), and 3.4 female green turtles (range = 2.7-4.5) nested annually. Because these calculations assume that the full complement of each female's egg clutches were laid at Curieuse, however, they would underestimate female numbers if there is significant movement of females between Curieuse and other islands. During our study, 88 known individual tagged hawksbills were encountered, some of which had been previously tagged at other sites in the inner island group, including Bird, Cousin, Cousine, Mahé, Praslin, and Ste. Anne islands, all within a distance of ~85 km from Curieuse (members of Turtle Action Group of Seychelles (TAGS), unpublished data). But the frequency of these inter-island movements has not yet been quantified.

We consider our four-season survey of nesting hawksbills at Curieuse to be a robust estimate of annual nesting activity (Table 2) given that the remigration intervals (the time between the consecutive nesting seasons of individual females) for hawksbills nesting in the inner islands of Seychelles are typically two to three years (Mortimer & Bresson 1999). In contrast, our data quantifying annual nesting activity and seasonality for green turtles are the first collected at Curieuse and represent only two full seasons. Remigration intervals for green turtles in the Inner Islands of Seychelles are unknown. We found that, on average, fewer than five female green turtles nested annually, with much variation between seasons (Fig. 3). In the Western Indian Ocean (WIO), green turtles typically nest during every month of the year, with seasonal peaks that vary between sites (Dalleau *et al.* 2012), between seasons and

even within the boundaries of a given site (Mortimer *et al.* 2011b; Mortimer 2012).

Our estimated average of 53.4 hawksbills nesting annually (Table 2) suggests a 50-100% increase in the nesting population since 1984 when an estimated average of 20-30 individuals nested each season (Mortimer 1984). Higher rates of population increase during the same three decades, however, have been recorded at nearby hawksbill rookeries where the nesting populations have been better protected over longer periods of time. At Cousin Island there was an eightfold increase documented between 1968 and 2008 (Allen *et al.* 2010), and at Aride Island a similar increase was recorded during the same period (Mortimer 2004; Island Conservation Society (ICS), unpublished data). This raises the question of why the rate of increase for Curieuse Island is only a fraction of that recorded at Cousin and Aride islands.

Likely explanations involve a combination of poaching, human disturbance and habitat limitations. Cousin and Aride islands were both designated as Special Reserves, in 1975 and 1979, respectively, which afford them the highest level of protection of any natural site in Seychelles. Turtles at Curieuse received legal protection in 1979, but heavy poaching continued until the mid-1990s (Mortimer 1984, 2004), and Curieuse is not yet free of poaching as indicated by the remains of three hawksbill turtles we encountered during the 2011-2012 nesting season.

Perhaps the most important issue, however, is the relatively unique vulnerability of the daytime nesting hawksbill populations of Seychelles to the negative impacts of human activity and disturbance, unsupervised tourism, and unregulated coastal development. These females, which nest almost exclusively in the daytime, will readily abort a nesting attempt when they detect movement (Mortimer 2004). In Seychelles, high-density hawksbill nesting beaches have only persisted where they are not subjected to high levels of human activity or coastal development. Diurnal hawksbill nesting is compatible with tourism at sites where tourist density is low (Mortimer 2004), *e.g.*, at Bird Island with 26 chalets and 5 km of nesting beach, at exclusive five star private islands that cater to low volume tourism, or in the Special Reserves where

tourist behaviour is strictly regulated. But, concentrated nesting has all but disappeared on the more densely populated islands of Mahé, Praslin and La Digue (Mortimer 1981, 2004; members of Turtle Action Group of Seychelles (TAGS), unpublished data).

Tourists to the Seychelles National Parks provide important revenue. Some 30,000 tourists visited Curieuse annually during 2007-2013 and each paid an entrance fee of approximately \$15 USD. At Curieuse, tourists are free to visit on any day of the week between 9 am and 5 pm (although visiting hours are rarely enforced); and they may access the island aboard a variety of watercraft including taxi boats, private yachts, and local tour boats. Their impacts on nesting turtles include disturbance from beach and boat traffic, swimmers and snorkelers, and pollution from lighting and noise. In contrast, tourists only visit Cousin and Aride islands on specially designated visiting days, and only as members of guided tour groups that are not allowed to roam unsupervised. Nesting activity at Curieuse was once more evenly distributed amongst the seven beaches (Mortimer 1984, 2004; Rulie 2002), but the high concentrations of tourism activity at Anse St. Jose and Baie Laraie have become incompatible with diurnally nesting hawksbills (Mortimer 2004). Almost 85% of the estimated 195 egg clutches laid annually at Curieuse are now concentrated on only 480 km of nesting beach at Grand Anse and Anse Papaie (at an average density of 34 clutches per 100 m per year). This demonstrates the significance of Grand Anse/Anse Papaie as a regional hotspot for turtle nesting, and highlights the importance of protecting this critical nesting habitat.

Currently, the Regulations of Curieuse National Park prohibit visitors from accessing Grand Anse and Anse Papaie. We commend the Government of Seychelles and SNPA for their efforts to protect this critical nesting habitat. But, there is no guarantee that it will remain intact for nesting turtles into the future. In Seychelles, there are fewer restrictions on development of land within a National Park than within Special Reserves. The current legislation for Curieuse under the National Parks and Nature Conservancy Act (1969) states that a National Park is “an area set aside for the propagation, protection and preservation of wildlife, or the preservation of places or objects of aesthetic, geological, prehistoric, historical, archaeological or other scientific interest, for the benefit, advantage and enjoyment of the general public.” Unfortunately, this does not exempt National Parks from commercial development. In theory, plans for development would have to be approved by the Seychelles National Environment Commission (NEAC), but the Minister has the final decision. There are no specific guidelines for important sea turtle nesting areas within the current legislation; and although the protected area policy of Seychelles is currently under review, no major changes are in motion regarding land development within National Parks.

We do not believe the current legislation provides the necessary safeguards for the nesting beaches at Curieuse under National Park status. We suggest that new legislation be adopted that allows for complete protection of key marine turtle nesting habitat, or that the status of Curieuse Island be re-classified in full or in part based on its national and international importance as nesting habitat for the Critically Endangered hawksbill. Indeed considering that Curieuse is not the only Seychelles National Park that hosts globally important turtle nesting habitat we believe it would be prudent to reassess the current legislation. The Ste. Anne and Silhouette National Parks both host hawksbill nesting populations of similar magnitude to

that of Curieuse and have had hotel developments and marinas proposed adjacent to critical nesting habitat. These issues highlight the inadequacy of Seychelles National Park legislation to propagate, protect and preserve wildlife.

Protection of nesting beaches is perhaps the most essential component of any sea turtle conservation program (Mortimer 2000). Our results showed that Curieuse may host as many as 80 individual nesting hawksbills annually along with a number of highly endangered Inner Island nesting green turtles. With this in mind we recommend the following actions:

- 1) Provide formal protection in perpetuity for the critical nesting habitat at Grand Anse and Anse Papaie. This should preclude any coastal development behind or on these beaches, and a minimum of human activity at the beach. Ideally, no boat traffic should be allowed within 400 m of this small stretch of coastline in order to minimize interference with turtle mating, internesting behaviour and hatchlings. But currently there is no legislation with which to regulate offshore activity in the vicinity of a National Park.

- 2) Continue to count tracks and tag turtles at all nesting beaches of Curieuse on a regular and consistent basis in order to assess long-term trends in the population and to provide surveillance against poaching. Annual and long-term monitoring is vital for documenting population trends.

- 3) Enforce rules that prohibit tourist access to Grand Anse and Anse Papaie. A vigilant Park Ranger at Baie Laraie would prevent access of tourists to adjacent Anse Papaie, especially at low tide when tourists typically try to approach the beach by sea.

- 4) Provide appropriately placed information boards that: a) inform tourists of restricted access zones; and b) describe a “Code of Conduct” for when in the presence of nesting turtles. Ensure Rangers and volunteers are available to explain the least intrusive way to view nesting turtles.

- 5) Keep all nesting beaches clear of obstacles, such as fallen trees and rubbish that impede turtle access to the beach platform, especially at Grand Anse and Anse Papaie. Fallen trees and branches downed by a violent storm in 2003 remain problematic at Grand Anse, and need to be removed.

In summary, we recognize that Curieuse National Park is one of the most important nesting sites for hawksbills and green turtles of the Inner islands of Seychelles. The Republic of Seychelles was recently named one of five small island nations declared world leaders in conserving threatened species (Rodrigues *et al.* 2014). The increase in nesting abundance described here for Curieuse and other nearby islands in the Praslin group is evidence of the effort dedicated to the protection and conservation of sea turtles in Seychelles by Government, parastatal organizations (*e.g.*, Seychelles National Parks Authority (SNPA), NGOs (*e.g.*, Global Vision International, Island Conservation Society, Nature Seychelles, and others), conservation scientists, and also community stakeholders. We highlight the need to protect critical turtle nesting habitats of national and global significance and to adjust national legislation accordingly.

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Fibropapillomas in a Loggerhead Sea Turtle (*Caretta caretta*) Caught in Almofala, Ceará, Brazil: Histopathological and Molecular Characterizations

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There are many threats to marine turtles, including habitat destruction, pollution, coastal development and fishing activities. Additionally, diseases such as fibropapillomatosis (FP), which is characterized by the development of skin tumors (Adnyana *et al.* 1997; Bugoni *et al.* 2001; Marcovaldi & Thomé 2000; Mast *et al.* 2005; Oravetz 2000) may directly or indirectly impact sea turtles. The prevalence tends to be higher in marine environments under the impact of human activities. Environmental pollutants such as organochlorine and organophosphate compounds, carbamates, selenium and heavy metals seem to be possible factors in the pathogenesis of FP (Aguirre *et al.* 1994; Aguirre & Lutz 2004; Chaloupka *et al.* 2009; Ene *et al.* 2005; Herbst 1994; Herbst & Klein 1995; Keller *et al.* 2014; Miao *et al.* 2001). Furthermore, tumors are most often observed when marine turtles are under stressful environmental conditions as well as when they are in regions with low water quality and in the presence of contaminants and toxins (Formia *et al.* 2007). It has been shown that green sea turtles (*Chelonia mydas*) use coastal areas more than other marine turtle species (Hirth 1997), which may explain why this species seems more susceptible than other sea turtles. Studies on the east coast of Florida in 1998 and 1999 compared and examined initial and recapture photographs; results indicated that 88% of recaptured green sea turtles showed regression of FP tumors (22 of 25 recaptured turtles). In Brazil, studies in the coastal region of Niterói, RJ, between July 2008 and August 2013, also documented fibropapilloma regression: 233 green sea turtles were captured and seven of them showed clear signs of regression of at least one tumor (Hirama & Ehrhart 2007; Tagliolatto 2013). Field workers have reported lesions like fibropapillomas in loggerhead sea turtles (*Caretta caretta*) from the Indian River Lagoon, Florida Bay and the Florida Keys (Florida, U.S.) and in Australia; olive ridley sea turtles (*Lepidochelys olivacea*) from the Pacific coast of Costa Rica and flatback sea turtles (*Natator depressus*) from Australia. Histopathological tests were confirmed in loggerhead and hawksbill sea turtles (*E. imbricata*; Ene *et al.* 2005; Herbst 1994).

Studies reveal that FP has multifactorial etiology in which several biological, genetic and environmental cofactors could play a significant role in the pathogenesis. Additionally, a viral etiology could be

at play, considering the alphaherpesvirus chelonid fibropapilloma-associated herpesvirus (CFPHV) (Ene *et al.* 2005; Herbst *et al.* 1998; Lackovich *et al.* 1999; van Houtan *et al.* 2010; Work *et al.* 2009).

The herpesvirus detected in fibropapilloma belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Scutavirus*, and is named chelonid herpesvirus 5 (ChHV-5). According to Herbst *et al.* (2004), phylogenetic analysis of ChHV-5 identified two major clades each with Atlantic and Pacific representatives. This herpesvirus has been identified in 100% of tumors induced by inoculation of tumor cell infiltrates (Ene *et al.* 2005).

In Brazil, many studies have been conducted to understand the physiopathology of FP. The aims of the present study were to characterize FP and detect CFPHV by histopathological and molecular analyses in tumors obtained from one loggerhead sea turtle caught at Boca da Barra, Ceará, Brazil.

A loggerhead sea turtle was captured incidentally by fishermen in fishing weir number 16 (Longitude: -39.82199°, Latitude: -2.89348°) at Boca da Barra, district of Almofala, Western Coast of Ceará, Brazil (Fig. 1) on 31 March 2010 (straight carapace length = 79 cm; Inconel flipper tag numbers BR66579 and BR66580; sex not

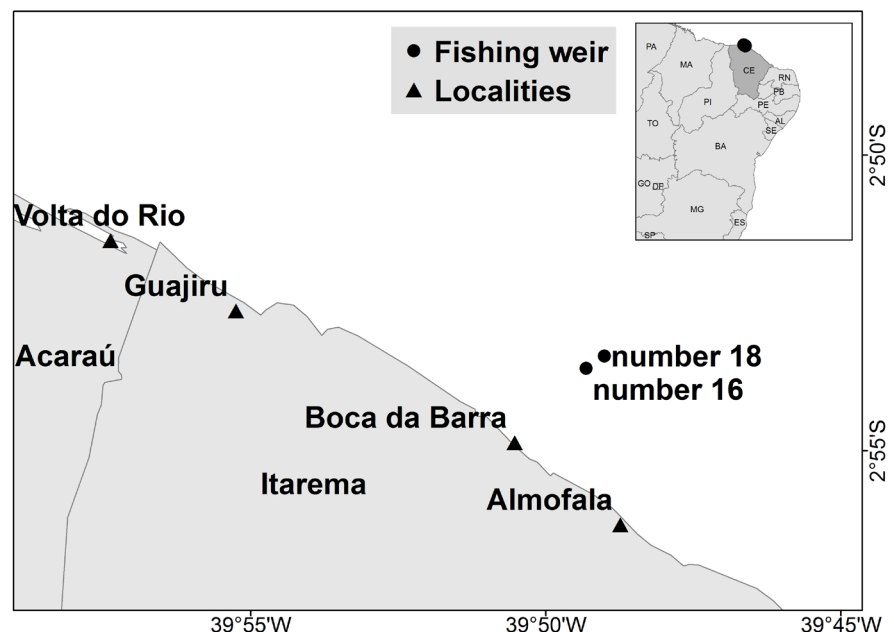


Figure 1. Fishing weirs number 16 and 18 in Boca da Barra, Ceará, Brazil, where the loggerhead sea turtle was captured.



Figure 2. Loggerhead sea turtle with tumors, captured at Boca da Barra, Almofala, Ceará, Brazil. Photo credit: Image bank of Projeto TAMAR/Ceará.

determined). After capture, the turtle was delivered to the Brazilian Sea Turtle Conservation Program (Projeto TAMAR-ICMbio), and released soon after. On 26 October 2012, the same loggerhead (84.6 cm SCL and 64 kg; classified as female) was recaptured at Boca da Barra in fishing weir number 18 (Fig. 1) (Longitude: -39.81682°, Latitude: -2.89007°) and cutaneous growths, similar to fibropapillomas found in green turtles, were observed. In this region, the fishing weirs are located at depths between 5 and 6 m (1-2 miles offshore) and set in an area where there are algae associated



Figure 3. Histopathological section of fibropapilloma from the loggerhead sea turtle stained using HE (obj. 4x). There is a great proliferation of epithelial cells characterized by ballooning degeneration (blue arrow), thickening of the stratum corneum (yellow arrow). Photo credit: Image bank of Research Group on Fibropapillomatosis in Sea Turtles.

with marine gravel. The Ceará coast of northeastern Brazil extends 573 km along the Equatorial South Atlantic Ocean. This area is characterized by low industrial development and is considered an important feeding area for green sea turtles. Observational reports of loggerheads, olive ridleys, hawksbills and leatherbacks (*Dermochelys coriacea*) exist for this area (Lima *et al.* 2007; Lima *et al.* 2013; Marcovaldi 1993). The major threat to marine turtles in this region is the high rate of incidental capture in fishing weirs, gillnets and trawl nets (Lima *et al.* 1999; Lima *et al.* 2013; Marcovaldi & Marcovaldi 1999).

The loggerhead turtle had thirteen tumors (Fig. 2), which were classified according to categories of size: A (<1 cm), B (1-4 cm), C (>4-10 cm) and D (>10 cm) (Work & Balazs 1999) and anatomic region. Three tumors were obtained for histopathological and molecular analyses: (1) collected from the right front flipper (category A), (2) obtained from the neck (Category D) and (3) collected from the left front flipper (non-classified). Before sample collection, the affected regions were cleaned with alcohol. Tumor biopsies were collected using a scalpel blade between the skin and tumor, with a margin of safety avoiding neoplasia recurrence. Povidine® solution was used for asepsis and the bleeding was controlled with gauze compress for several minutes. Tumor biopsies were fixed in 10% neutral buffered formalin for histopathology analysis and subsequently stored in 70% alcohol and frozen at -20 °C until molecular processing. Slides of the tumor samples were stained with hematoxylin-eosin (HE) and prepared at Laboratório de Histologia, Departamento de Patologia, Faculdade de Medicina

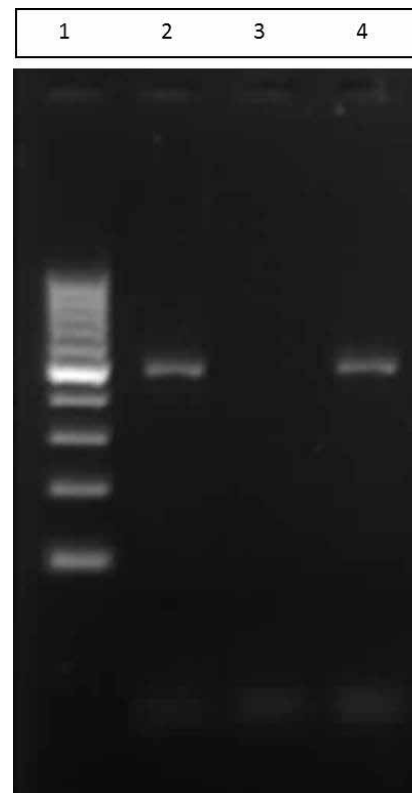


Figure 4. Electrophoreses in 1.5% agarose gel for 480-bp fragments using GTHV 2/GTHV 3 primers. (1) Ladder 100-bp; (2) Positive control; (3) Negative control; (4) Sample of fibropapilloma from *C. caretta*, positive for DNA polymerase of turtle herpesvirus.

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DNA extraction was carried out according to Chomksinsky (1993). The herpesvirus variant reactions were conducted using methods described by Ene *et al.* (2005). A 2.5- μ L DNA aliquot was submitted to PCR in a final 25- μ L reaction using the specific primers for DNA polymerase of turtle herpesvirus, GTHV2 (5'- GACACGCAGGCCAAAAAGCGA-3') and GTHV3 (5'-AGCATCATCCAGGCCACAA-3'), described by Quackenbush *et al.* (2001). The conventional PCR reaction was conducted in 12.625 μ L ultrapure water, 2.5 μ L of buffer solution for PCR (20 mM of Tris-HCl pH 8.4 and 50 mM of KCl), 4.0 μ L dNTP (200 μ M each dNTP), 1.25 μ L of each primer (0.4 μ M of each primer), 0.75 μ L of 1.5 mM MgCl₂ and 0.125 μ L of the enzyme Platinum Taq polymerase (Invitrogen Life Technologies). The sample was denatured at 94 °C for 5 min and then was amplified with 35 cycles (94 °C for 30s, 62 °C for 30s, 72 °C for 30s) and then a 10-min cycle at 72 °C in a thermal cycler. The amplified product in all PCRs was resolved by electrophoresis in 1.5% agarose gel in Tris-borate-EDTA buffer (0.045 M Tris-Borato, 0.5 M EDTA) and a voltage of 1-10 V/cm of gel. The PCR product was a fragment ~480 bp and was purified using a GFX Purification kit (GE Healthcare, UK). The purified product was submitted to an automated sequencing reaction performed using a commercial kit: ABI Prism Big Dye TM terminator - Cycle Sequencing Ready Reaction (Applied Biosystems, CA). The nucleotide sequence was processed using the BioEdit program and aligned with Clustal W (Thompson *et al.* 1994) and the sequence will be submitted to GenBank (www.ncbi.nlm.nih.gov). The molecular analysis was carried out at Applied Molecular Biology and Serology Laboratory, Departamento de Medicina Veterinária Preventiva e Saúde Animal, FMVZ-USP.

The fibropapilloma tumors varied in their appearance, such as color (white, pink and gray) and texture (smooth to verruciform). On microscopic analysis, proliferative lesions in epithelial cells characterized by ballooning degeneration varying from minimal to extensive were observed. Orthokeratotic hyperkeratosis with thickened *stratum corneum* was also verified in formations (Fig. 3). In the basal layer, it was found that vacuolated cells were necrotic and the *stratum spinosum* showed vacuolated cells often related to the underlying basal layer process. Furthermore, a highly vascularized hyperplastic stroma consisting of connective tissue resulting in an increase of dermal thickness was also seen. Wide proliferation of fibroblasts was found in all sections, mainly in the papillary area. Also, we verified a diffuse infiltration of mononuclear cells in the dermis. Many of these characteristics were also described in previous studies, which reported fibropapillomas in *C. mydas* and *E. imbricata* (both species captured on the Brazilian coast), in oral and skin tumors from *C. mydas* caught in the Hawaiian Islands and in *C. mydas* from the Indian River Lagoon, Florida, U.S.A. (D'Amato & Moraes-Neto 2000; Jacobson *et al.* 1989; Work *et al.* 2004; Zwarg *et al.* 2014).

According to histopathological examination, the cutaneous growths correspond to papillomas or fibropapillomas according to their epithelial and/or stromal proliferation. The presence of herpesvirus was confirmed with the agarose gel, and the amino acid sequence of the fibropapilloma sample suggested that it was a fragment of DNA polymerase from ChHV-5, according to GenBank (Fig. 4). However, new DNA amplification and sequencing has been

conducted for the molecular characterization of ChHV-5, because some parts of the antisense sequence presented low quality in the electropherogram analysis. The molecular characterization of alpha-herpesvirus in marine turtles is part of a study conducted by the Research Group on Fibropapillomatosis in Sea Turtles - USP.

Fibropapillomas are less common in loggerheads, so more studies about the herpesvirus variants are needed for this species. Rodenbusch *et al.* (2012) detected the ChHV-5 in a fibropapilloma of a green turtle, caught on the coast of Rio Grande do Sul, RS-Brazil; this first report formalized the characterization of this variant in green sea turtles found in that area. Our study enabled us to characterize the tumors in the sampled loggerhead sea turtle and to detect if the herpesvirus variants were the same ones as those found in green sea turtles. This information adds knowledge about fibropapillomatosis in loggerheads that use the coast of northeastern Brazil.

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Hopper Dredging Impacts on Sea Turtles on the Northern Coast of Rio de Janeiro State, Brazil

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The northern coast of the state of Rio de Janeiro, eastern Brazil, is an important nesting ground for loggerhead turtles (*Caretta caretta*), with about 1500 nests laid annually (Lima *et al.* 2012). It also hosts foraging grounds for juvenile green turtles (*Chelonia mydas*) and serves as a migration corridor (and possibly provides foraging habitat) for olive ridley turtles (*Lepidochelys olivacea*) (Reis *et al.* 2010; TAMAR - Brazilian Sea Turtle Conservation Program database, unpublished data) and leatherback (*Dermochelys coriacea*) turtles (López-Mendilaharsu *et al.* 2009).

Despite the high importance of the area for sea turtles, construction began in 2008 for a large, private mixed-use port complex, named Açú Superport. This enterprise, which is the largest port-industry facility in South America, is located at 21.8157°S, 41.0060°W (Fig. 1), just south of the city of São João da Barra and about 260 km (geodesic distance) from the city of Rio de Janeiro (Barreto & Quinto Junior 2012). The port complex, now in operation, is equipped with two sets of terminals, one offshore and the other onshore, which together have 17 km of wharves accommodating up to 47 vessels

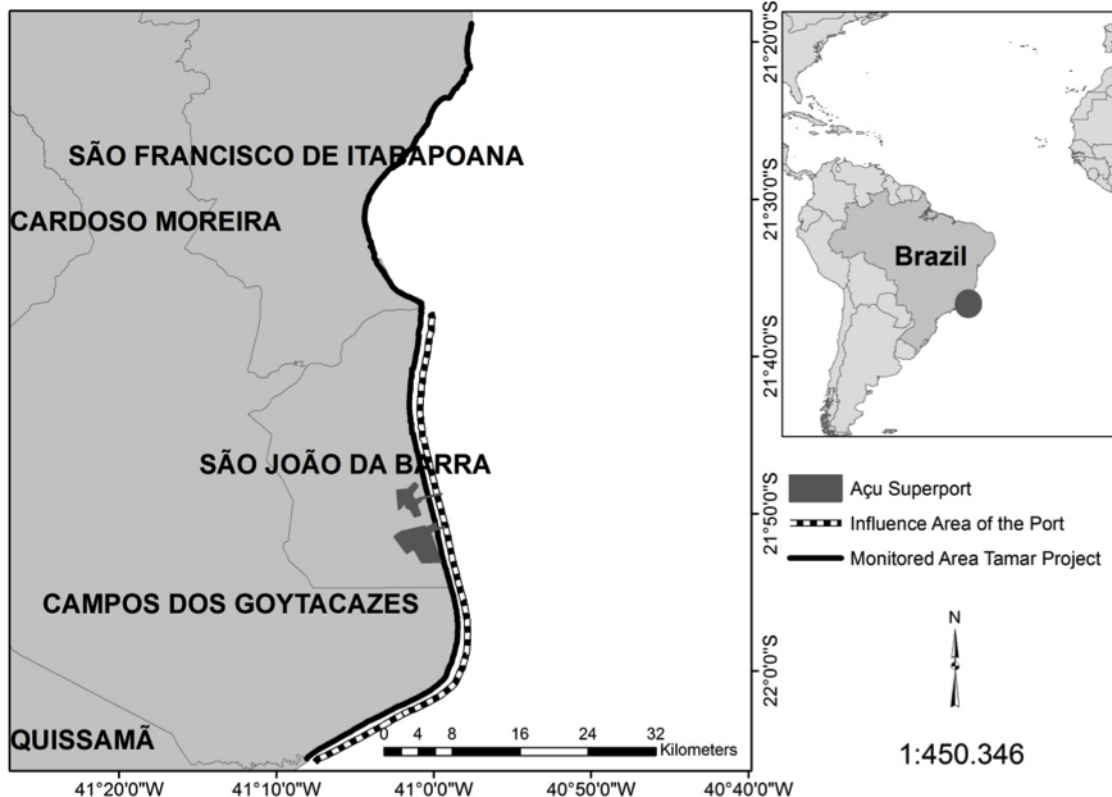


Figure 1. Map showing the location of Açú Superport and shipyard (in dark gray); the area of influence near the Açú Superport monitored by the port operator under TAMAR supervision (dotted line) and the overall area monitored by TAMAR (black line), which is an important nesting ground for loggerheads.



Figure 2. Dead adult loggerhead cut in half by a hopper dredge on the northern coast of Rio de Janeiro state, Brazil.

(Ditty & Rezende 2014). Here we provide an account of sea turtle mortalities observed in the region that appear to be the result of dredging operations during both port construction and operation.

Since the beginning of construction in 2008, hopper dredges have been used for clearing and maintaining access channels, a turning basin, and a harbor basin, all to facilitate navigation of vessels using the port. In 2012, additional hopper dredges entered into operation for the construction of a new terminal and shipyard. Because the port complex is situated in a high-energy coastal zone, maintenance dredging of constructed channels is regularly required to remove sediments that build up after being transported and deposited by currents.

Hopper dredging was first identified as a source of turtle mortality in 1980, when 71 turtle interactions with hopper dredges were recorded over a period of five months in Canaveral Channel, Florida (NMFS 1991; Dickerson et al. 2004). Subsequently, between 1980 and 2003, 508 turtles have been impacted by dredgers from 38 different locations throughout the southeastern United States (Dickerson et al. 2004). Hopper dredges remove bottom sediments through articulated suction pipes, discharging it into a holding area (hopper) within the vessel. The dredged material is then taken away from the dredged area and subsequently released in a disposal area. During active dredging operations, the hopper dredge dragheads are



Figure 3. Dead adult leatherback cut in half by a hopper dredge on the northern coast of Rio de Janeiro state, Brazil.

slow-moving and nearly silent while suctioning bottom sediments, thereby potentially causing injuries or death to sea turtles that are entrained into the draghead (Dickerson et al. 1991; Banks & Alexander 1994; Dickerson et al. 2004; Fitzpatrick et al. 2006). Besides physical harm (e.g., massive injuries, fractures, crushed tissues and hemorrhage) and mortality, indirect impacts such as alteration or destruction of foraging habitat might also occur, especially when dredged material is placed on rocky bottom habitats commonly used by sea turtles as foraging grounds. In addition, dredging may stir up toxic pollutants that have settled and become trapped by bottom sediments. Common measures used to reduce the likelihood of turtle and hopper dredger interactions include: working during times of year when turtles are less likely to occur at the project location; using deflectors and specially designed dragheads; relocating turtles from the project area via net capture prior to dredging operations (Dickerson et al. 2004).

In the case of the Açú Superport complex, only the dredges used in the construction of the shipyard and onshore portion of the complex were equipped with sea turtle deflectors and had observers on board to detect any turtle interactions, for reasons related to the environmental licensing process. Dragheads were checked after every load to ensure that no sea turtles had been entrained and turtle deflectors were also inspected to assure correct alignment. Daily

	0-21 cm	22-41 cm	42-61 cm	62-81 cm	82-101 cm	<101 cm	NM	Total
CC	0	0	0	2	3	3	18	26
CM	0	7	1	0	2	0	58	68
DC	0	0	0	0	0	3	1	4
LO	0	0	1	3	0	0	7	11
NI	0	0	0	0	0	0	3	3
Total	0	7	2	5	5	6	87	112

Table 1. Sea turtles with dredging-related injuries, per species and size class category, found stranded along the area monitored by the port operator under TAMAR supervision, on the northern coast of Rio de Janeiro state, Brazil, from 2008 to 2014 (CC = *Caretta caretta*; CM = *Chelonia mydas*; DC = *Dermochelys coriacea*; LO = *Lepidochelys olivacea*; NI = not identified; NM = not measured).



Figure 4. Dead olive ridley cut in half as a result of a hopper dredge interaction on the northern coast of Rio de Janeiro state, Brazil. Note the presence of the dredge in background.

inspection reports were filed by the observers, summarizing the dredging events of the day and documenting cases when a turtle was sighted or a lethal take occurred. Since dredging activities began, two different deflector types have been used: rigid deflectors and flexible chains. Although the rigid deflector is more effective in reducing entrainment than the flexible deflector (Nelson & Shafer 1996), the hopper dredge operator decided to use the latter.

Additional monitoring was conducted daily along 66 km of adjacent coastline, by the port operator, under Projeto TAMAR supervision. Each stranded turtle found (dead or alive) was identified, photographed and measured with a flexible tape. Curved carapace length (CCL) was measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudal scutes. Curved carapace width (CCW) was measured across the widest part of the carapace, perpendicularly to the longitudinal body axis (Marcovaldi & Laurent 1996).

Fresh dead turtle carcasses found on the beach were taken to a laboratory for necropsy and decomposing carcasses were examined in the field by veterinarians. Both the necropsies and the field examinations were performed to determine whether the observed injuries were the result of dredge interactions and also to differentiate dredging lesions from propeller injuries, which are typically multiple linear-parallel lacerations and/or fractures that may penetrate the skin, coelomic cavity or the skull. The correct identification of dredging injuries was based on comparisons with lesions recorded in our 20-year stranding database, and the review of publications related to dredging, and technical reports provided by a pathologist from the local university (Universidade Estadual do Norte Fluminense), who has extensive experience in sea turtle injuries caused by hopper dredges. Injuries to sea turtles from hopper dredges are caused by blunt force trauma and are generally characterized by serious crushing wounds (*e.g.*, extensive fractures, lacerations and amputations). Only individuals with these types of injuries were considered in this evaluation.

Between 2008 and 2014, 1725 stranded sea turtles were found along the 66 km coastline monitored by the port operator under Projeto TAMAR supervision. This monitoring was part of a mitigation environmental measure required by the licensing

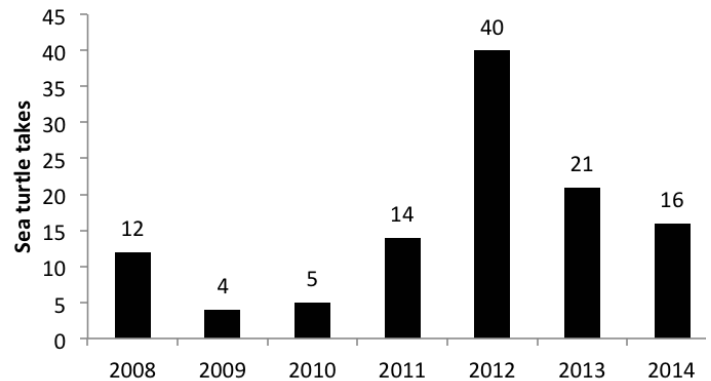


Figure 5. Sea turtle mortalities by hopper dredging, per year, along the northern coast of Rio de Janeiro state, Brazil.

authority. Of all stranded sea turtles observed, 112 individuals were found with injuries indicative of dredging interaction, including two that were found entrained in the hopper dredge draghead by observers. Of these, 68 were green turtles, 26 were loggerheads, 11 were olive ridleys, four were leatherbacks and three could not be assigned a species identification.

Considering that hopper dredges have huge dragheads and strong suction power, interactions with sea turtles frequently result in fatal injuries. The 112 turtles with dredging-related injuries often were cut in half and/or had parts of their carapace and/or flippers missing (Figs. 2, 3 and 4). Because most of the 112 turtles had parts of their carapace missing (Figs. 2, 3 and 4), only 25 of them could be accurately measured (Table 1).

In addition to the analysis of stranded turtles, we also evaluated dredging operations, by reviewing technical reports or by direct field observation of the dredging events. The technical reports included information regarding the number of hopper dredges operating within a defined period of time. Whenever these reports were unavailable, dredging activities were documented through direct observation and information available in the environmental license of the port. Although dredging operations started in 2008, we had access to data on hopper dredge activities only at the shipyard, which started in the end of 2011. Therefore, we lack precise information on the total number of dredges (port + shipyard) operating from 2008 onwards, and on the exact number of days/month that the dredges from the port were operating between 2008 and 2014.

The available information on dredging activities from 2011 onwards was cross-referenced with sea turtle takes and stranding location data, to search for a potential correlation between dredge events and sea turtle mortality. For this analysis, we considered two situations: periods with dredging activity and periods without dredging activity. We found that strandings with injuries directly attributable to hopper dredging operations occurred more frequently while dredging was active (Figure 6). Only 11 stranded turtles occurred in periods without dredging operations. However, even these occurrences may be related to hopper dredging, as we do not know exactly when the dredges from the port were operating. Additionally, months with <10 days of dredging activities were considered as months without dredging operations.

Individuals were classified as juveniles or adults based on CCL measurements, considering the minimum values for nesting females in Brazil (see Kotas *et al.* 2004; Silva *et al.* 2007; Thomé *et al.* 2007; Sales *et al.* 2008; Grossman *et al.* 2007; Marcovaldi and Chaloupka,

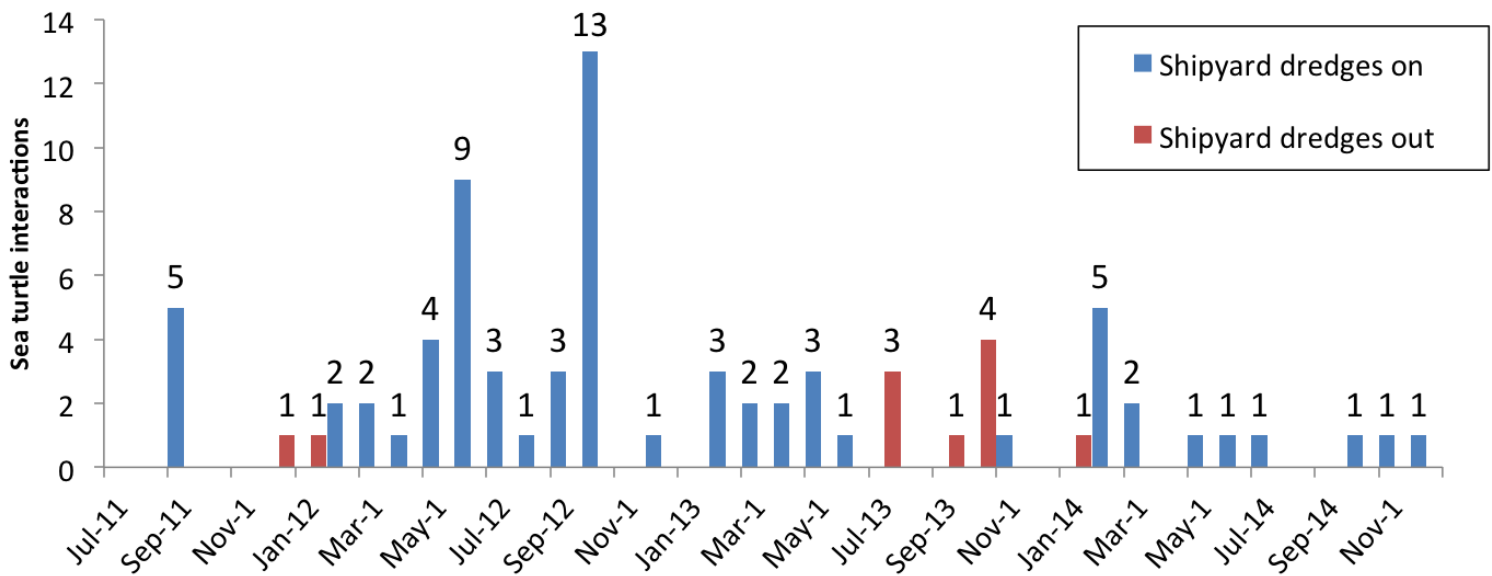


Figure 6. Sea turtle interactions (y axis) by dredging operation situation per month (x axis) along the area of influence of the Açu Superport, from September 2011 to December 2014. Red columns mean no dredging operations in those months. Blue columns indicate active dredging operations in those months. From October 2011 to January 2012 and from July 2013 to September 2013, the shipyard dredges were not operating; however, we do not have information on the port dredges.

2007; Santos et al. 2010 for reference values). All except two of the loggerheads were adult-sized (Table 1), reflecting the fact that the northern coast of Rio de Janeiro is a nesting area for loggerheads. Sixty-three green turtles were juveniles (Table 1), reflecting the importance of the area as foraging ground for juvenile *C. mydas*. Almost all leatherbacks and olive ridleys taken by the hopper dredge were adults and subadults (Table 1). The northern coast of Rio is considered a high use area for adult leatherbacks (López-Mendilaharsu et al. 2009) and an important migration corridor for adult olive ridleys (TAMAR, unpublished data).

In 2012, the number of turtles with these injuries increased considerably (Fig. 5), probably because additional hopper dredges started operating in the region during the construction of the new terminal and shipyard. In 2013 and 2014, we noted a gradual decrease in dredged-related strandings. The cause(s) for this decreasing trend are not clear, but may be related to the partial adoption of mitigation measures, or a shift to dredging closer to the shore.

In order to minimize dredging impacts on sea turtle populations, Projeto TAMAR has provided technical support to the environment agencies in charge of the port operation, for the development of a detailed plan to prevent additional incidental takes. Mitigation measures such as alternative dragheads, deflector equipment, as well as environmental time windows and using dredges other than hopper dredges, have been proposed, following Dickerson et al. (2004), with an understanding that the effectiveness of each measure is dependent on local environmental conditions. However, the Açu Superport authority chose not follow all of the proposed mitigation measures. For instance, a no-dredge environmental time window during the entire nesting season, which extends from October to March, was proposed but not implemented. Since November 2012, dredging has been restricted only to nighttime hours from November to January. However, subsequent numbers of observed stranded turtles linked to dredging operations in November-January remain similar to levels prior to November 2012 (Fig. 6). Had all the proposed mitigation

measures been adopted and properly carried out, at both the port and the shipyard, it is possible that the dredging impacts on sea turtles in the area would have been much reduced.

According to Koch et al. (2013), the probability that turtles which are injured or killed in the water and subsequently are found on the beach as a stranded animal varies widely and usually does not exceed 10-20% of total mortality. Therefore, it is likely that the number of incidental captures by hopper dredges in this area based on stranded animals is an underestimate. Additionally, given the powerful draw of water into the hopper dredge during active dredging, a turtle entrapped on the underside of the draghead would never free itself while the pumps are on. While on the bottom, the massive draghead could pulverize a turtle beyond recognition (Dickerson & Nelson 1990).

Hopper dredging poses a serious threat to sea turtles, and on the northern coast of Rio de Janeiro, dredges have been killing turtles at different life stages, including gravid females, which have a high reproductive value for the larger population, because they are able to contribute new offspring to future generations. Considering these findings and what has been learned so far, even with proper application of all mitigation measures, we strongly discourage hopper dredging operations on sea turtle nesting grounds during nesting seasons. Additionally, in areas of high sea turtle concentration (e.g., foraging grounds), care must be taken to ensure that there is minimum impact on these animals and other marine species. In high-density areas, it is recommended that hopper dredging operations should be carried out only if appropriate mitigation measures are sufficiently adopted, including but not limited to: the use of sea turtle deflector dragheads, intake and overflow screening, sea turtle relocation (away from the path of the dredge) and onboard observers. We hope our observations and recommendations will be used to assist future dredging project proponents and environmental agencies, in the selection of safe and appropriate mitigation measures.

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REPORT

Report of International Symposium on Research/Conservation and Future Prospects of Sea Turtles in Taiwan

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An International Symposium on Research/Conservation and Future Prospects of Sea Turtles in Taiwan was held 15-16 June 2015 in Kaoshiung, Taiwan. Research on sea turtle biology and conservation in Taiwan was initiated by Forestry Bureau, Council of Agriculture, in 1992 and has been carried on by the Marine Ecology and Conservation Laboratory, Institute of Marine Biology of National Taiwan Ocean University, led by Professor I-Jiunn Cheng. During the past three decades, we have conducted long-term studies on the nesting ecology of green turtles on Wan-an Island of Penghu Archipelago (since 1992), Liuchiu Island of Pingtung County (since 2001) and Lanyu Island of Taitung County (since 1997). In addition, we have also conducted other sea turtle research, including work on satellite telemetry, nesting physiologies, nest site selection, population genetics, etc. However, with rapid development of modern technology and networks, much new knowledge, concepts and tools are continuously available. If we fail to learn about these new techniques and information, we will not be able to keep pace with the modern scientific societies. In view of this, we decided to take the opportunity of 23 years of sea turtle research and 20 years since the establishment of the sea turtle refuge site on Wan-an Island, to hold the "International Symposium on Research/Conservation and Future Prospects of Sea Turtles in Taiwan." For this meeting, we invited 10 international experts/scholars including four from Japan (Prof. Naoki Kamezaki, Dr. Yoshimasa Matsuzawa, Prof. Katsufumi Sato, Dr. Hideo Hatase), one from Malaysia (Dr. Nicolas J. Pilcher), three from Florida, US (Prof. John Weishampel, Ms. Nicole Montgomery, Ms. Adrienne McCracken), one from Italy (Dr. Sandra Hochscheid), and one from China (Director Ho-Xiang Gu) to share their valuable research and field experiences with the students and public audience. This allowed us to understand the current trends on international sea turtle research, and conservation and management policies. Ninety-six people registered for the

symposium, including schoolteachers, students, veterinarians, NGOs, members of a Buddhist group and governmental officials.

At the meeting, the head of the Forestry Bureau, Council of Agriculture introduced the current status of sea turtle in Taiwan. Professor Cheng and his PhD student introduced the sea turtle research and stranding network in Taiwan. The foreign participants made presentations on various topics, including the global and regional conservation strategies of the IUCN Marine Turtle Specialist Group, the operation and achievement of sea turtle volunteer systems in Japan, the core work of a sea turtle protected site in China, the application of geographic information system (GIS) to assess the impact of light pollution on nesting beaches, the influence the incubation temperature to hatchling sea turtle physiology, the use of biologgers to study the behavior of sea turtle in the ocean, the relationship between the life history strategies and spatial distribution of sea turtles in the ocean, an overview of the nesting beach work and rehabilitation actions of a privately funded sea turtle center in Florida, etc. These presentations will help shape and inform future avenues of research on sea turtles in Taiwan, which was one of the goals of the symposium. After the meeting, all international participants were invited to visit the unique coral reef of Liuchiu Island in Pingtung County, southern Taiwan, where about 160 resident green turtles inhabit in the nearshore waters. All participants used snorkeling gear to observe the behavior of green turtles in the water, and gain a better understanding of the sea turtle resources of Taiwan.

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