

Marine Turtle Newsletter

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Photos by Michelle Dorantes and Luis Sequeira.

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The Ocean Foundation
c/o Marine Mammal and Turtle Division
Southwest Fisheries Science Center, NOAA-NMFS
8901 La Jolla Shores Dr.
La Jolla, California 92037 USA
E-mail: mtn@seaturtle.org
Fax: +1 858-546-7003

Matthew H. Godfrey
NC Sea Turtle Project
NC Wildlife Resources Commission
1507 Ann St.
Beaufort, NC 28516 USA
E-mail: mtn@seaturtle.org

Managing Editor:

Michael S. Coyne
SEATURTLE.ORG
1 Southampton Place
Durham, NC 27705, USA
E-mail: mcoyne@seaturtle.org
Fax: +1 919 684-8741

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Beach Crabbing as a Possible Hindrance to Loggerhead Marine Turtle Nesting Success

Emily M. Drobos^{1,2}, Matthew Ware¹, Valencia K. Beckwith¹ & Mariana M. P. B. Fuentes¹

¹Department of Earth, Ocean, and Atmospheric Science, Florida State University, Tallahassee, FL 32306, USA

(E-mail: emd14@my.fsu.edu, mw15w@my.fsu.edu, vkb14@my.fsu.edu, mfuentes@fsu.edu);

²Florida Fish and Wildlife Conservation Commission, Tallahassee, FL 32399, USA

Marine turtles are impacted by a multitude of threats at both their terrestrial and oceanic life stages (Wallace *et al.* 2011; Fuentes *et al.* 2015). At nesting grounds, impacts are generally from artificial light (Witherington 1992; Silva *et al.* 2017), coastal development (Fuentes *et al.* 2016; Nelson Sella & Fuentes 2019), coastal armoring (Rizkalla & Savage 2011), beach nourishment (Brock *et al.* 2009), poaching (Koch *et al.* 2006), predation (Lutcavage *et al.* 1996), marine debris (Duronslet *et al.* 1991; Beckwith & Fuentes 2018; Garrison & Fuentes 2019), and climate change (Janzen 1994; Hawkes *et al.* 2009; Fuentes *et al.* 2011; Hamann *et al.* 2013). The presence of humans during marine turtle nesting can also disturb nesting, causing marine turtles to return to the water or even stop them from emerging onto land (Witherington 1992; Jacobson & Lopez 1994). Although less documented, recreational catch-and-release of ghost crabs, *Ocypode quadrata*, (*i.e.*, “beach crabbing”) in coastal areas is an activity commonly conducted on beaches in the northern Gulf of Mexico which may influence marine turtle emergence and nesting decisions. Beach crabbing usually consists of one person or a group of several people with flashlights walking up and down the beach chasing ghost crabs with nets, usually accompanied by noise. To explore whether marine turtle nesting is affected by beach crabbing, we investigated the spatial extent of beach crabbing and marine turtle activities on St. George Island, Florida, one of the most important nesting sites for the Northern Gulf of Mexico Loggerhead (*Caretta caretta*) Recovery Unit.

Tourism is an important industry on St. George Island: tourist numbers average over 136,000 total visitors per year, and an estimated 113,400 of those visitors stay overnight on the island

(Blair pers. comm. 2017). During the summer, 81.8% of the homes are seasonally occupied with visitors rotating on a weekly basis (Fuentes *et al.* 2016). From 2014 to 2017, the study region has had, on average, 312 ± 171 SD nests per year across the nesting season with peak nesting occurring in mid-June (Becker pers. comm. 2018). To determine the exposure of nesting turtles to beach crabbing and potential implications, we conducted nighttime surveys along 17.5 km of the nesting beach at St. George Island (Fig. 1) on foot between 2100 hrs and 0400 hrs during the peak of the nesting season in 2017: 10 June to 19 June. The study area included the southwestern and middle sections of the island. The southwestern end contains a gated community with access restricted to residents and guests (Fig. 1). The middle of the island is accessible to all visitors with the main access point located at the lighthouse close to the bridge from the mainland. There are 17 additional smaller access points concentrated around the middle of the island (Fig. 1, Beach Access St. George Island, Florida 2018). The gated community on the southwestern end of the island enforces rules against noise and lights at night to mitigate human impacts on the beach ecosystem (<http://www.stgeorgeplantation.com/page/37437~701534/Rules-Regulations>). The survey area excluded the state park at the north end of the island as this area is not publically accessible at night.

During our surveys, the time and GPS location of any loggerhead activities (nests and false crawls) were recorded in addition to any beach crabbing activities and the number of people involved, as well as the number of flashlights used and the color of the light. Based on the time of the observation, marine turtle and human activities were grouped into one of two operational time categories: 2100

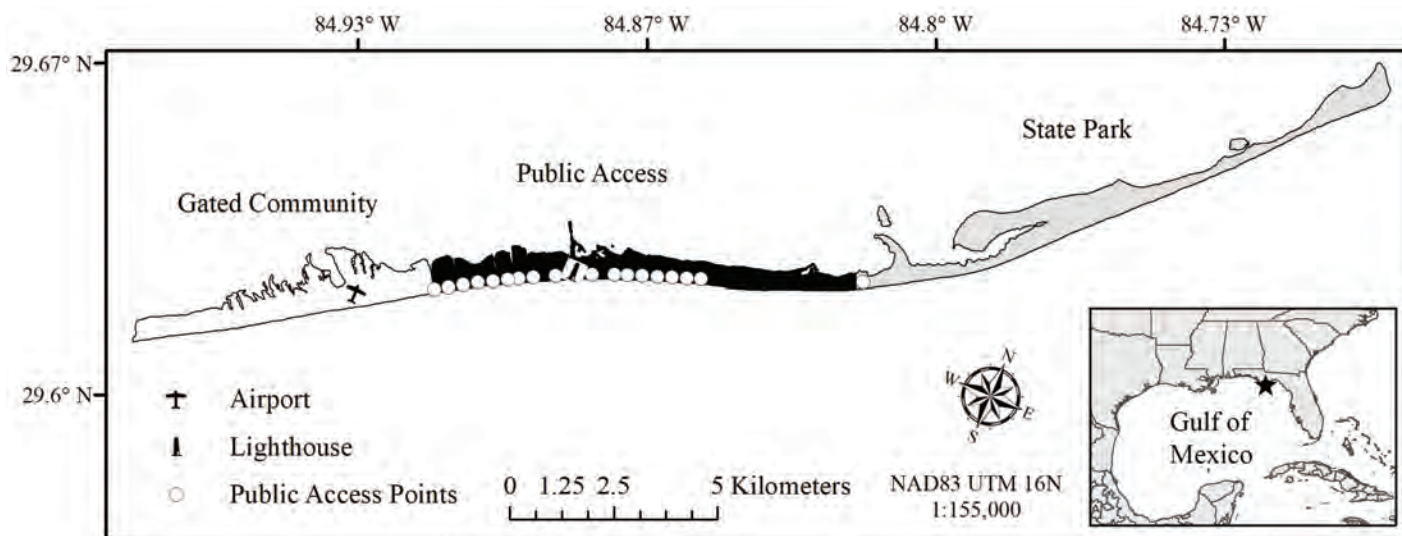


Figure 1. St. George Island, Florida, United States. This study included the gated community (southwest) and public access (middle) sections of the island. The State Park was not included in this study as it is not publically accessible at night.

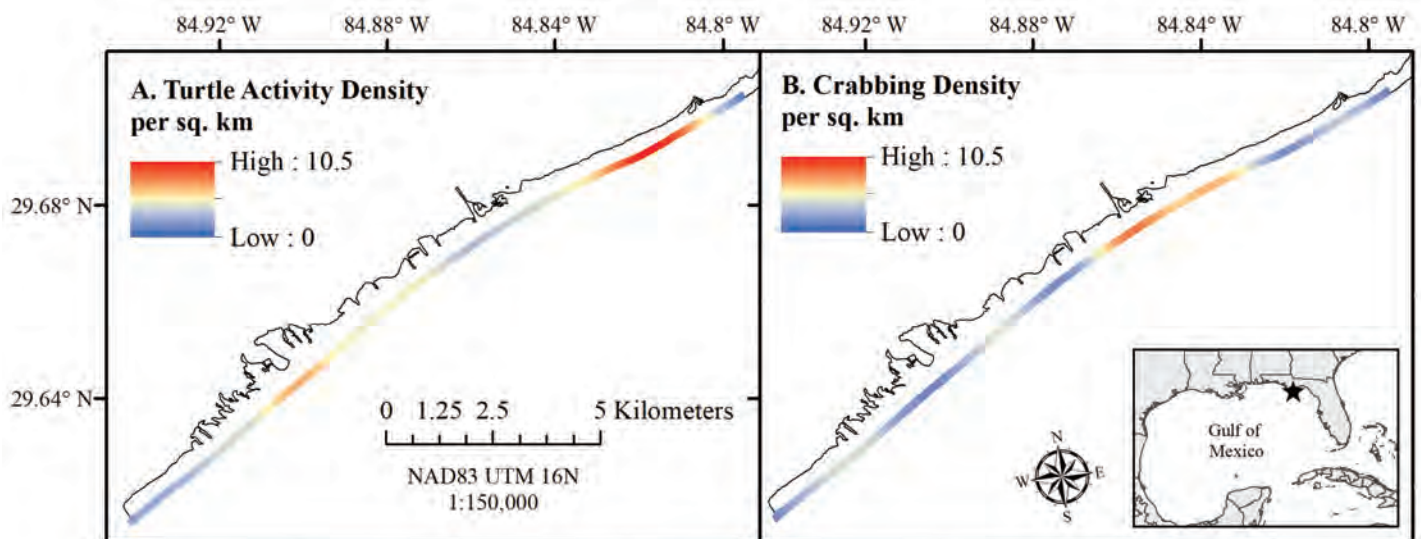


Figure 2. Kernel density estimates of all loggerhead turtle activities (A) and beach crabbing events (B) from 10 to 19 June 2017 at St. George Island, Florida.

to 2359 hrs and 0000 to 0400 hrs, if an event overlapped between both time periods, it was categorized based on the time the event was first observed. If a turtle was encountered during the surveys, researchers minimized their potential disturbance to the turtle by maintaining a safe distance from the turtle during her inbound crawl and nesting attempt, minimizing noise, and using red light only as needed, as per permit regulations. Differences in nesting success by time category were assessed by binomial generalized linear models. Kernel density estimates of turtle and beach crabbing activities were used to visualize distribution patterns. All analyses were run in R version 3.5.0 or ArcMap 10.3 (ESRI 2014).

During the surveys, there were a total of 117 loggerhead turtle activities (50 nests and 67 false crawls) in the study area with an overall nesting success of 42.7%. Most of the recorded turtle activities occurred after 0000 hrs (65.0%), with no significant difference in nest success ($p = 0.840$) between times. By comparison, 94 beach crabbing events were documented before 0000 hrs with an average of 6.0 ± 4.0 SD people using 4.6 ± 4.1 SD white lights and 0.1 ± 0.7 SD red lights per event. Only six beach crabbing events were recorded after 0000 hrs with an average of 2.7 ± 1.4 SD people using 2.7 ± 1.4 SD white lights and no red lights per event. Kernel density maps indicate that during the survey period, the greatest concentration of marine turtle activities were located just southwest of the state park and the lowest densities were near the lighthouse (mid-island, public access area) and southwest end of the island within the gated community (Fig. 2A). The greatest density of crabbing activity was located near the lighthouse while the lowest density was adjacent to the airport (Fig. 2B). This spatiotemporal pattern of turtle emergence may indicate that marine turtles at the study site may be avoiding times and locations of increased human nocturnal activity such as beach crabbing. Indeed, we did not observe any interaction between crabbers and turtles.

Avoidance of human activities is not the only reason why a female turtle may emerge later in the night to nest. Nesting may occur any time of night (Caldwell 1959) and a turtle may choose to abandon a nesting attempt for many reasons including distance from the nearest human settlement (Kikukawa *et al.* 1998, 1999), artificial

lighting (Raymond 1984; Proffitt *et al.* 1986; Witherington 1992), sand temperature (Stoneburner & Richardson 1981), improper beach substrate (Mortimer 1990), and poor weather conditions (Hughes *et al.* 1967), in addition to human disturbance (Dodd 1988). Although no formal lighting or topographical surveys were conducted as part of this study, artificial lights on St. George Island during the survey period could be seen from far distances as the beach's wide, flat berm and short dunes do not restrict residential or handheld lights. No major changes in sand grain size, scarp formation, or other geological characteristics along the length of the study site exist which could explain the distribution of turtle activities noted during the nighttime surveys. Marine turtles may time their emergence with the high tide to decrease the risk of nest inundation during incubation and to minimize energy expenditure (Frazer 1983, Wilson 2018). Though high tide did occur later in the night during the survey period, the small tidal range at St. George Island (0.37 m) means that turtles in the study area are unlikely to use high tide timing as a significant emergence cue (Frazer 1983).

Further research across the full nesting season and at additional beaches should be conducted to evaluate whether a causal relationship exists between beach crabbing and marine turtle nesting activities. Bustard *et al.* (1975) and Limpus (1985) showed that loggerheads approach and observe the beach from shallow water before coming onshore. This is the time that they are most sensitive to disturbance and they will retreat if they observe perceived danger. The movement of people and the use of bright white lights near the high water mark associated with beach crabbing activities could potentially be discouraging turtles from emerging from the water (avoidance behavior) or cause turtles to return to the water without completing a nest (Jacobson & Lopez 1994; Silva *et al.* 2017). Although prevalent on beaches along the Gulf coast of Florida, the exposure and impact of beach crabbing on turtles has not been previously quantified nor identified as a major issue.

Human leisure activities on beaches are increasing, intensifying pressures on these environments and the species that use them (Schlacher *et al.* 2008). As St. George Island is a marine turtle nesting site where houses are seasonally occupied, with a turnaround

of tourists every week during the nesting season, it may require outreach and education efforts to be structured specifically to engage with seasonal residents and elicit their support for conservation actions (Fuentes *et al.* 2016). One approach to minimizing the potential impacts of beach crabbing on marine turtles is to develop and advertise the use of turtle-friendly crabbing kits. These could contain red lights and a pamphlet explaining tips to reduce impacts to nesting marine turtles such as use of red lights instead of white lights, the need to minimize noise, and instructions to minimize interactions with a nesting turtle. Additional education efforts could include visible, short guides in rental homes and improved signage at beach entrances on how to minimize disturbances to nesting female turtles.

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Sand Temperature, Sex Ratios, and Nest Success in Olive Ridley Sea Turtles

Mead R. Binhammer^{1,2}, Maddie Beange² & Randall Arauz^{2,3}

¹Buzzards Bay Coalition, 114 Front St., New Bedford, MA 02745, USA (E-mail: mbinhammer@gmail.com);

²CREMA, Calle 114, 1.5 Km Norte, Barva, Heredia, 40201 Costa Rica (E-mail: madeleine.beange@gmail.com);

³Fins Attached Marine Research and Conservation, 19675 Still Glen Dr., Colorado, CO 80908, USA (E-mail: rarauz@finsattached.org)

Small changes in incubation temperature may have large effects on reptile egg development (Crews *et al.* 1994). Thus, it is important to consider fine scale, environmental variation at nest sites, as well as weather patterns and the effects of climate change when evaluating temperature effects on the condition and success of reptile nests (Mrosovsky & Yntema 1980; Kolbe & Janzen 2002). As climate change causes sea level rise, increases global temperatures, alters rainy and dry seasons and increases the proportion of severe weather events (Hawkes *et al.* 2009), sea turtle nests will face many new obstacles in the incubation environment.

In sea turtle species, high nest temperatures are associated with decreases in incubation period length, hatch success and locomotor performance (Bustard & Greenham 1968; Valverde *et al.* 2010; Fisher *et al.* 2014; Booth 2017). Lethal incubation temperatures, which can result in embryonic death and the loss of entire nests, vary between sea turtle species. The threshold point in olive ridleys (*Lepidochelys olivacea*) is considered to be 35 °C (Valverde *et al.* 2010; Hill *et al.* 2015). As regional temperatures increase, the chance of nest temperatures surpassing this threshold also rises. Incubation temperatures also play an important role in sex determination (Mrosovsky & Yntema 1980; Standora & Spotila 1985), so population demography can be affected by the sand temperatures in which embryos develop (Kaska *et al.* 2006). Increased incubation temperatures produce higher female to male hatchling sex ratios, a trend that is becoming more pronounced with the continuation of anticipated increases in global temperature (Mrosovsky & Yntema 1980; IPCC 2007; Rogers 2013).

These effects could be particularly severe in western Central America, as the region may be exceptionally susceptible to climate change (Giorgi 2006). Future effects on the area could include warming, increased number of hurricanes and tropical storms, and stronger effects of the El Niño Southern Oscillation (ENSO) (Giorgi 2006). The impacts on ENSO are of significant importance to the region as they are predicted to result in higher sea surface temperatures and less precipitous rainy seasons, followed by periods of drought (Waylen *et al.* 1996; Santidrián-Tomillo *et al.* 2012). Other marine reptiles have adaptation strategies to survive these climatic anomalies (Wikelski & Thom 2000), though the same adaptations have not been demonstrated in sea turtles (Hawkes *et al.* 2009). While sea turtles may be able to adapt strategies to survive gradual environmental changes, by processes such as shifting nesting phenology, it is unlikely these mechanisms will be effective in the fast-paced and far-reaching impacts of current climate change. As the effects of ENSO coincide with the August-January nesting season of olive ridleys on the Pacific coast of Costa Rica (Dornfeld *et al.* 2014), climate change may have a dramatic impact on population size and nesting levels.

The olive ridley is one of the smallest species of sea turtle and is

listed as Vulnerable on the IUCN Red List (Abreu-Grobois & Plotkin 2008). It is also the most abundant sea turtle species worldwide, with its largest rookeries located along the northeast coast of India and the western coasts of Central and North America (Polovina *et al.* 2004). Olive ridleys engage in a bimodal nesting strategy, with females participating in arribada (mass-arrival) nesting events, or, as is found in other species of sea turtle, solitary nesting (Valverde *et al.* 2010). During a breeding season, females emerge from the surf to lay a clutch of eggs on approximately 1-3 occasions (Hamel *et al.* 2008). Each of these clutches has 80-120 eggs and is deposited in a roughly 35 cm-deep cavity, with an incubation period of 45-50 days (Pandav *et al.* 1998).

Using a long-term dataset, we examined relationships between sand and in-nest temperatures and olive ridley emergence success,



Figure 1. Location of the study site on the western coast of the Nicoya Peninsula in Guanacaste, Costa Rica.

Nest	Year	Temperature Average (°C)	Nest Success (%)	Hours Above 35°C	Incubation Period (Days)	2nd Trimester Average Temperature (°C)
C1	2013	31.9	97.8%	67	50	31.4
C2	2013	32.6	72.9%	96	48	32.5
C3	2013	32.5	85.6%	25	46	33.2
C4	2014	34.2	44.8%	341	49	34.2
C5	2014	33.5	66.7%	191	46	33.0
C6	2014	30.6	85.7%	0	53	29.0
C7	2014	28.6	90.7%	0	56	28.1
C8	2015	34.6	5.1%	374	47	34.5
C9	2015	32.9	90.5%	6	46	33.2
C10	2015	31.5	86.7%	0	47	31.0
C11	2015	30.8	90.7%	0	51	29.9
C12	2015	30.7	76.5%	0	50	30.0
C13	2016	32.5	70.7%	60	47	33.1
C14	2016	32.1	85.7%	193	47	30.2
C15	2016	32.5	63.7%	237	49	31.5
C16	2016	31.3	88.2%	0	49	32.2
C17	2017	32.0	87.6%	58	48	31.6
C18	2017	31.5	84.6%	6	50	31.7
C19	2017	31.0	95.9%	167	50	30.8
C20	2017	30.8	94.4%	37	49	30.8
SM1	2013	31.9	85.6%	43	48	31.0
SM2	2013	32.6	69.7%	177	46	31.9
SM3	2013	32.7	76.0%	132	45	32.3
SM4	2013	31.5	94.1%	0	49	30.7
SM5	2013	31.1	86.8%	0	50	31.1
SM6	2013	33.2	76.9%	196	45	33.3
SM7	2014	33.3	91.0%	73	44	33.2
SM8	2014	33.4	96.0%	46	46	32.8
SM9	2014	32.6	95.0%	81	47	32.1
SM10	2014	32.2	94.0%	67	49	32.3
SM11	2014	30.5	98.0%	0	53	29.4
SM12	2014	31.3	87.0%	58	51	30.0
SM13	2015	34.2	68.0%	348	47	34.0
SM14	2015	31.4	93.0%	0	49	32.0
SM15	2015	31.6	90.0%	0	47	31.6
SM16	2015	30.4	83.0%	0	52	29.6
SM17	2016	31.3	86.0%	0	49	30.9
SM18	2016	32.4	86.0%	15	47	32.6
SM19	2016	31.6	85.0%	0	49	32.3
SM20	2017	30.7	92.0%	0	56	30.6
SM21	2017	29.6	99.0%	0	61	29.5

Table 1. Summary table of nest data by associated nest code. Nest codes beginning with C are from Playa Costa de Oro, while nest codes beginning with S are from Playa San Miguel.

hatchling sex ratios and incubation periods in a solitary nesting population on Playa Coyote, Costa Rica. While arribada populations of olive ridleys are relatively better documented, this study provides novel information relevant to many locations where the species nests in a solitary fashion.

Study site. Playa Coyote, encompassing Playa Costa de Oro and Playa San Miguel, is located in Nandayure, Guanacaste, Costa Rica (Fig. 1). Both beaches have a turtle conservation project under the direction of CREMA (Rescue Center for Endangered Marine Species). On Playa San Miguel, this conservation project began in 1998, while the project on Playa Costa de Oro started in 2012 (Viejobueno & Arauz 2015; Beange & Arauz 2017). Both projects involve nightly patrols to locate nesting sea turtles and relocate nests to a hatchery to alleviate poaching pressures. Primarily olive ridley turtles nest on Playa Coyote, however occasional leatherback (*Dermochelys coriacea*), green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles also come ashore. The monitoring season coincides with the peak-nesting season for olive ridleys on the Nicoya Peninsula, beginning at the start of June and concluding in mid-late December.

Research Techniques. To record sand temperature in the on-beach environment, three HOBO-model pendant-series temperature loggers (Onset, USA), programmed to log temperature hourly, were placed at depths of 25-30 cm below the surface of the sand in order to mimic the mid-nest depth of a natural olive ridley nest. These three loggers were placed at each of the following positions: above the vegetation line (~100% vegetation cover), at a midpoint between the vegetation and the beach (~50% vegetation cover) and on the beach (~0% vegetation cover). In-nest temperatures were also recorded with the use of a similarly programmed HOBO data logger placed at the midpoint (50% of all eggs were placed in the chamber, followed by the logger and remaining eggs) of an olive ridley clutch within the hatchery. The number of these in-nest temperature loggers varied from year to year, with anywhere from 2-6 per year being used on both Playa Costa de Oro and Playa San Miguel between the nesting seasons of 2013-2017 (Table 1). In addition, two HOBO data loggers were placed at depths of 25-30 cm outside of nests within the hatchery to collect sand temperature data throughout the incubation period and control for the thermal production of the incubating eggs. The temperature data collected by the HOBO

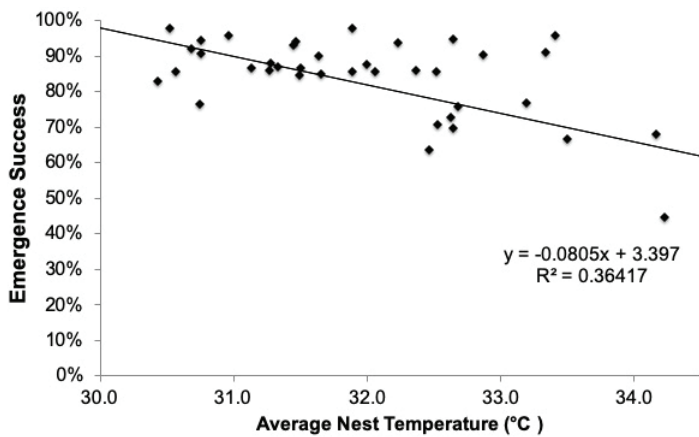


Figure 2. Linear regression for average nest temperatures over the incubation period and emergence success; $n = 41$ and $P \leq 0.001$, with the resulting 95% confidence interval for the slope = -0.046, -0.115.

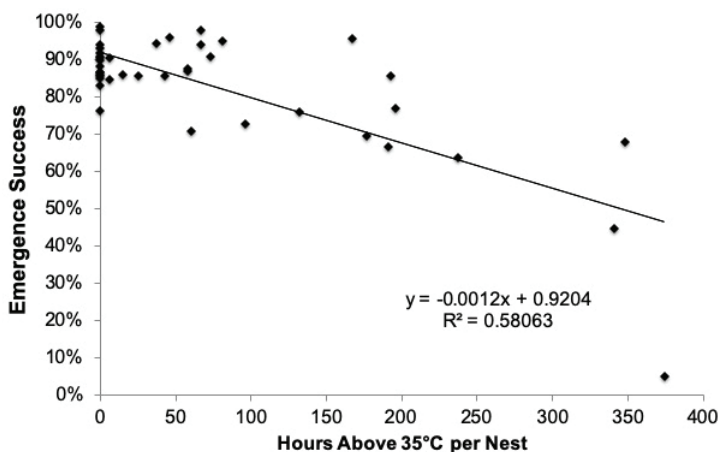


Figure 3. Simple linear regression for hours per nest exceeding 35 °C and emergence success, with $n = 41$, $P \leq 0.001$, and the 95% confidence interval for the slope = -0.00087, -0.0015.

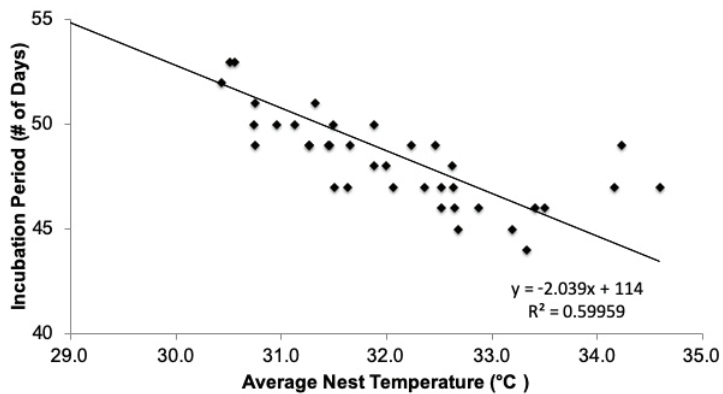


Figure 4. Simple linear regression for the incubation period of nests related to the average nest temperature in °C, with $n = 41$, $P \leq 0.001$, and the 95% confidence interval for the slope = -1.499, -2.579.

loggers were then analyzed by either trimester averages for in-nest HOBOs, or monthly and yearly averages for beach and out-of-nest hatchery sensors. The number of hours each nest exceeded the lethal field incubation temperature of 35°C was also recorded (Hill *et al.* 2015). Data were recorded to delineate the dates in which the data loggers were placed and when the majority of hatchlings emerged, in order to determine incubation periods.

Upon hatching, each nest was excavated to determine hatching and emergence success, and the developmental stage of embryos in eggs that failed to hatch. The incubation period was defined as the date eggs were placed in the hatchery to the date of the major emergence of hatchlings. Trimesters were calculated by dividing the incubation period (in days) by three. Wibbels (2007) reported the thermo-sensitive period of sex determination to fall within the second trimester of incubation, and the pivotal temperature to lie between 30.0-31.0 °C, so we assumed the pivotal temperature was 30.5 °C in this study, and estimated sex ratios using the average temperature of the second trimester of each nest (Valverde *et al.* 2010). Average temperatures of the second trimester above 30.5 °C were considered to be majority-female nests (greater than 50% female), and majority-male nests were presumed for temperatures below 30.5 °C. While pivotal temperatures may vary geographically and among species, we used similar techniques to those employed by Valverde *et al.* (2010) on Ostional Beach, the more proximate location studying sex ratios and temperature in olive ridleys (Hernández-Echeagaray *et al.* 2012; Rogers 2013).

Prior to emergence, plastic mesh was placed on top of the nests so that emerging hatchlings could be corralled and counted. Hatching success was calculated by adding the number of emerged hatchlings to the number of hatchlings found in the nest chamber during excavations and subsequently dividing this sum by the total number of eggs counted at the time of deposition. Linear regression models and associated correlation calculations, including Pearson correlations, linear regression t-tests and 95% confidence intervals for slope, were used to test relationships between average nest temperature and both emergence success and incubation period. These calculations were also used to test the association between emergence success and the number of hours each nest exceeded the 35 °C lethal temperature threshold. All calculations were performed in Microsoft Excel.

Hatchery and Beach Sand Average Temperatures. Temperature data were recorded from both the hatchery and beach environment on Playa Coyote. Monthly average hatchery and beach temperatures did not show significant correlations to emergence success (hatchery $R^2 = 0.17$, $P = 0.06$). As anticipated, incubation period was correlated with out-of-nest hatchery sand temperature ($R^2 = 0.03$, $P = 0.01$).

Nest Temperatures. Average nest temperatures from Playa San Miguel and Playa Costa de Oro for the whole incubation period ranged from 28.6 to 34.6 °C (Table 1). Nests that experienced any hours above 35 °C ($n = 25$) had a significantly lower average emergence success (78.9%, $P = 0.03$) than nests that did not experience a single hour above 35 °C (89.1%, $n = 16$). The nest that experienced 374 hours above 35 °C had an emergence success of 5.1%, much lower than the average of 84.8% in the 40 other nests studied. Emergence success was correlated with nest temperature ($R^2 = 0.36$, $P < 0.001$), and hours exceeding 35 °C ($R^2 = 0.58$, $P < 0.001$) (Figs. 2 & 3). Incubation duration was also correlated with nest temperature ($R^2 = 0.60$, $P < 0.001$) (Fig. 4).

We estimated that 78.6% of all nests in both Costa de Oro and San Miguel from 2013-2017 resulted in female-biased hatchling sex ratios. Overall temperature can dramatically impact sex ratios, incubation periods, mortality during incubation and hatching success in species of sea turtles (Matsuzawa *et al.* 2001; Wibbels 2007; Hawkes *et al.* 2009; Valverde *et al.* 2010; Hill *et al.* 2015). Our results show that an increase in temperature can increase female hatchling production and decrease emergence success. This is of substantial importance due to the potentially strong impact of climate change on Costa Rican temperatures and weather conditions in the coming years and decades (Giorgi 2006). While the effect of this female bias on the population is unknown, it is worth noting the potential impact of climate change on olive ridley demography. Given that as little as a 2-3°C increase in nest temperature was shown to significantly decrease incubation period and emergence success in this study, the impacts of climate change on the nesting environment is worth rigorous study. Extremely high temperatures during incubation have been associated also with reduced locomotor performance and fitness of hatchlings, which may affect on their survival outside of the nest environment (Maulany *et al.* 2012; Fisher *et al.* 2014; Booth 2017). It is important to note that all nests monitored for temperature were located within a hatchery environment and therefore not subject to the impacts of vegetation, wind-erosion and other on-beach conditions that could affect temperature and emergence success. Our results suggest that the consistent monitoring of incubation temperatures, both within and outside of the hatchery environment, is imperative to learn and respond to the impacts climate change and temperature anomalies may have on hatchling production and their sex ratios.

On top of predicted climate change impacts in Costa Rica, ENSO can exacerbate changes in both temperature and precipitation patterns (Waylen *et al.* 1996; Santidrián-Tomillo *et al.* 2012). ENSO can cause periods of drought followed by extended, elevated air temperatures, which can have a great impact on sea turtle nesting. Breeding numbers of green turtles in Australia, for instance, are correlated with ENSO trends, due to the variation in foraging resources associated with sea surface temperature fluctuations (Limpus & Nicholls 2000). Additionally, as our study suggests that nest temperature is negatively correlated with emergence success, and that emergence success is impacted by the number of hours above 35°C, temperature anomalies such as ENSO are of great importance to conservation efforts of olive ridleys in Costa Rica.

The correlation between extremely high temperatures and lower emergence success suggests that peak temperatures, along with more traditionally monitored trimester and incubation averages, may be imperative to predicting the hatch success and condition of each olive ridley nest. The R^2 values reported here are the result of 41 data points and suggest the need for continual study of nest response to incubation temperatures. A longer-term dataset is needed to confirm the relationships found in this study, such as temperature's negative correlation with hatchling production. We observed only three nests that had average temperature >33.5 °C, thus further study of nests at this high temperature is warranted. In addition, more study is needed to determine the impacts of ENSO on sand temperatures and moisture in the future. Due to the influence of the hydric environment on sex determination (Lolavar & Wyneken 2015), weather events such as ENSO may impact nests both by temperature fluctuations and precipitation patterns. With

olive ridleys' bimodal nesting strategy, the study of emergence success and sex ratio production in arribada nesting populations is also imperative. As some arribada populations have been shown to experience higher incubation temperatures than solitary nests laid at the same time (Honavar *et al.* 2008; Dornfeld *et al.* 2015), the impacts on sex ratios and hatchling production are of extreme importance, especially in regards to ENSO and climate change.

The data presented here support a common concern for sea turtle conservation projects: an increasing feminization of hatchlings (Booth & Astill 2001; Wibbels 2007). We did not verify the sex of each hatchling, instead electing to use estimates, and therefore may have conservatively estimated a female-biased sex ratio of 78.6%. This estimates provide a sense of the sex ratio production from nests, although more complicated mechanisms may be at work in sex determination, such as timing of peak temperatures and moisture content of the sand (Rogers 2013; Lolavar & Wyneken, 2015). The IPCC (2007) suggested a potential increase in temperature of 3.5 °C by the year 2100; thus, sex ratios of sea turtle species worldwide may be impacted significantly. Sand temperature manipulation such as shading and watering (Hill *et al.* 2015) may be necessary tools to counter the impacts of climate change on populations that experience extremely high temperatures. As Costa Rica may be particularly susceptible to the impacts of climate change (Giorgi 2006), and some olive ridley populations along the Pacific coast have been shown to exceed lethal temperature thresholds during incubation (Valverde *et al.* 2010; Dornfeld *et al.* 2014), sand temperature manipulation in this region may be compulsory to ensure hatchling production and mitigate further female bias. As many conservation projects in the area use hatcheries in response to poaching pressures, the infrastructure exists to employ and monitor shading and watering techniques on the ground.

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Breeding Biology of Green Sea Turtles Stranded in Potiguar Basin, Northeastern Brazil

Marília Anielle da Silva Fabrício^{1,2}, Aline da Costa Bomfim^{1,3,4}, Silmara Rossi^{1,5},
Daniel Solon Dias de Farias^{1,3,4}, Raquel S. Cavalcante^{1,2,3}, Hamilton Barroso Mourão Junior^{1,3},
Flávio José de Lima Silva^{3,6} & Simone Almeida Gavilan^{1,3}

¹Universidade Federal do Rio Grande do Norte, Centro de Biociências, Departamento de Morfologia, Laboratório de Morfofisiologia de Vertebrados, Brazil (E-mail: mariliaanielle@yahoo.com.br; alinebonfim_7@hotmail.com; smara.rossi@gmail.com; danielsolon_@hotmail.com; raquelmarinho.souza@gmail.com; haamiltonjnr@gmail.com; gavilansimonealmeida@gmail.com); ²Programa de Pós-graduação em Biologia Estrutural e Funcional, Universidade Federal do Rio Grande do Norte, Centro de Biociências, Brazil; ³Projeto Cetáceos da Costa Branca, Universidade do Estado do Rio Grande do Norte, Laboratório de Monitoramento de Biota Marinha, Brazil; ⁴Programa de Doutorado em Desenvolvimento e Meio Ambiente - PRODEMA, Universidade Federal do Rio Grande do Norte, Brazil; ⁵Grupo de Estudos sobre Fibropapilomatose em Tartarugas Marinhas, Universidade de São Paulo, Brazil; ⁶Universidade do Estado do Rio Grande do Norte, Laboratório de Monitoramento de Biota Marinha, Brazil (E-mail: flaviogolfinho@yahoo.com.br)

Juvenile green sea turtles can be found along the Brazilian coast accounting for the higher number of occurrences (strandings, sightings and bycatch), and in pelagic areas along the northeast coast (Sales *et al.* 2008; Santos *et al.* 2011). In Brazil, their principal nesting areas are the Island of Trindade, Espírito Santo (mean curved carapace length or CCL of nesting females = 116.8 cm), Atol das Rocas Biological Reserve, Rio Grande do Norte (mean CCL = 115 cm), and Fernando de Noronha Archipelago, Pernambuco (Moreira *et al.* 1995; Bellini *et al.* 1996; Bellini & Sanches 1996; Grossman *et al.* 2003). Previous studies predicted their age at maturation of 25-50 years in Australia (Chaloupka *et al.* 2004); from 35 to >50 years in the Hawaiian Islands (Balazs & Chaloupka 2004); and 30-44 years (28 years were required to reach the minimum size) in Florida, Costa Rica and Mexico (Goshe *et al.* 2010).

Green sea turtles can be considered prepubescent when they reach 50-73 cm, straight carapace length, pubescent at 71-79 cm, and mature at 82 cm (Miller & Limpus 2003). Data on carapace length of the smallest recorded size for nesting females can be used to estimated size at sexual maturity (Marcovaldi *et al.* 1999; Sanches *et al.* 1999). However, carapace length is not a suitable measure for an accurate identification of an individual's maturation stage, such as prepubescent individual, pubescent individual or subadult, non-breeding adult, breeding adult and senescent adult (Miller 1997; Miller & Limpus 2003; Pérez *et al.* 2010).

The absence of dimorphic sexual chromosomes in marine turtles precludes sex determination through genetic methods, and there is no sexual dimorphism in juvenile green turtles (Godley *et al.* 2002; Rosa 2009). Various techniques have been applied to determine the sex of immature marine turtles, such as radioimmunoassay, laparoscopy, and ultrasonography (Wibbels 2003; Ceriani & Wyneken 2008). According to Otsuka *et al.* (2008), there is limited knowledge about histological characterization of testes and epididymis of juvenile green sea turtles, and the information available is insufficient to provide a detailed understanding of the gonadal changes in sea turtles. Since then, Rosa (2009) analyzed gonad samples from both sexes of juvenile green sea turtles in Brazil, and suggested that characteristics based on texture and adherence of gonads are not adequate for sex classification in individuals with 27.5-68 cm CCL. Pérez *et al.* (2010) analyzed gonads from Cuban green and hawksbill (*Eretmochelys imbricata*) sea turtles in different stages of development (prepubescent, pubescent and mature), and verified

that ovarian follicular development is an essential process in the classification of maturation stages associated with size. However, we found few studies focused on histological features of gonads, most research focused on nesting areas that provide knowledge on marine turtle breeding biology, including nesting behavior, interseasonal nesting movements, number of nests, size of nester and clutch, egg size and hatching success (Hirth 1980; Bjorndal & Meylan 1983; DeGroot & Shaw 1993; Garduño-Andrade *et al.* 1999; Wang & Cheng 1999; Grossman *et al.* 2003; Moura *et al.* 2014).

Studies on the gonadal histology of sea turtles will serve to improve methodology in assessment of sexual maturity, and reinforce conservation efforts, such as long-term management programs involving researchers, government, non-governmental organizations, and local community; regional management plans for coastal fishing activity, which play a role and an impact on the marine turtle populations. Our aims were to: (1) determine the sex ratio of green turtles stranded in Potiguar Basin, Brazil, identifying their stages of sexual maturity based on macro- and microscopic examination of the gonads; and (2) correlate these stages of maturity to the size of examined individuals.

This research was carried out in northeastern Brazil, between Caiçara do Norte (5.06699° S; 36.07678° W) in Rio Grande do Norte state (RN) and Icapuí (4.64674° S; 37.54780° W) in Ceará state (CE) covering an approximately distance of 300 km (Fig. 1). This region is known as the Potiguar Basin and it is considered an important feeding ground for green turtles (Gavilan-Leandro *et al.* 2016; Farias *et al.* 2019). Since 2010, the Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN) in Brazil has conducted the Beach Monitoring Program in the Potiguar Basin (*Programa de Monitoramento de Praias da Bacia Potiguar* - PMP-BP). The PMP-BP is part of an environmental constraint compliance required by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) associated with the oil exploitation operated by PETROBRAS (Petróleo Brasileiro S.A., Agreement Number 2500.005657510.2).

Our research was based on data obtained between 2011-2015, with daily monitoring conducted by a trained field team of PCCB-UERN. A portable GPS was used to record the location of both live and dead stranded green turtles. Live turtles were taken to a rehabilitation center in Areia Branca/RN and were evaluated clinically by veterinarians. Individuals found to be mildly or

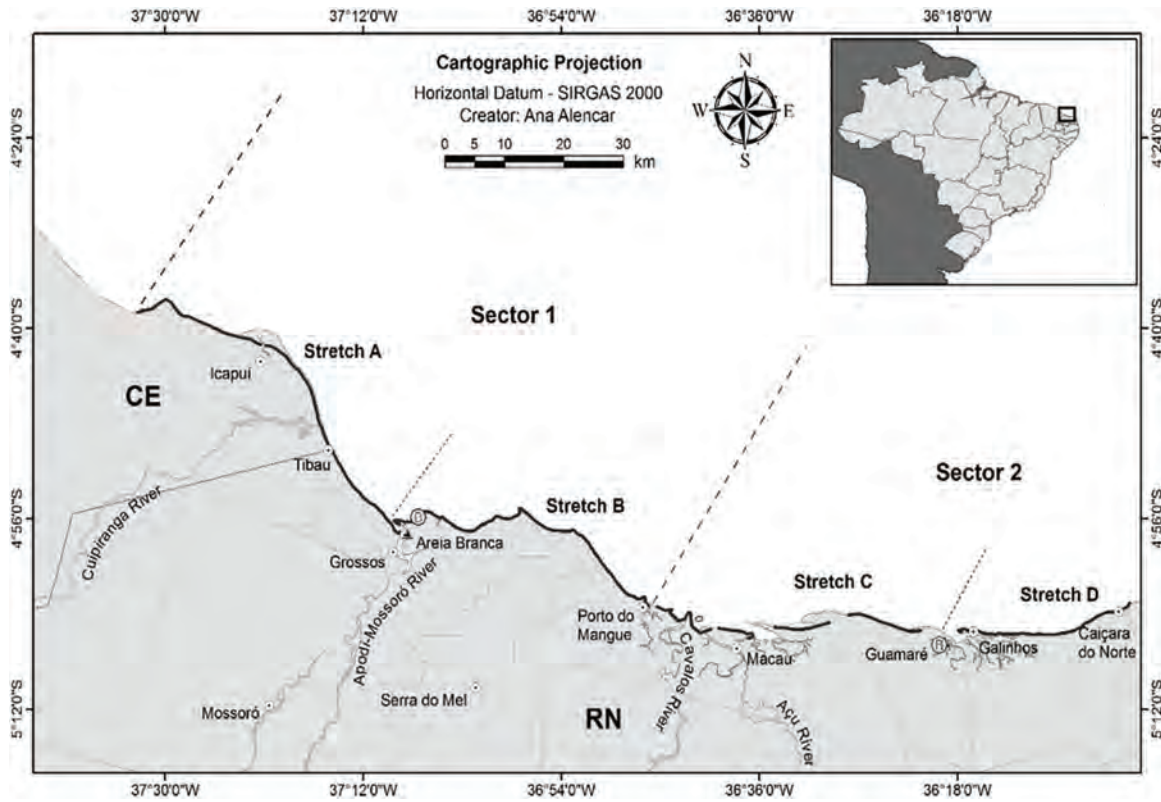


Figure 1. Geographical distribution of the study area. Source: Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN). RN: Rio Grande do Norte, and CE: Ceará.

moderately decomposed (condition codes D2 and D3, Flint *et al.* 2009) and those that died during rehabilitation were necropsied, and representative gonad samples were obtained and fixed with 10% formalin. Gonad samples from 78 green turtles (53 females, CCL 29-107 cm and 25 males, CCL 27-114 cm) were dehydrated, diaphanized and embedded in paraffin, and serial 5 μ m sections were prepared and stained with hematoxylin-eosin (HE).

We recorded CCL from nuchal to notch between supra-caudal scales, following Bolten (1999), using a flexible tape and green sea turtles were binned according to the following size classes (cm): 20-39.9, 40-59.9, 60-79.9, 80-99.9, 100-119.9, and 120-139.9. The sex of examined individuals was determined through macroscopic (visual) analysis of the gonads during necropsy. Based on Ceriani & Wyneken (2008), we analyzed texture, adherence, shape, and presence of paramesonephric ducts. We also observed external morphological characteristics that determine sexual dimorphism in the adult individuals: males have smaller CCL and larger front flipper claws than females; and also have curvature in their forelimbs and long prehensile tails, with the cloacal opening in the terminal portion of the tail (Wyneken 2001; Godley *et al.* 2002). Through microscopic examination, attention was given to the maturation stages based on characteristics of germinative cells present in the gonads. The stages of female maturation observed followed Miller & Limpus (2003) and reports from Pérez-Bermúdez *et al.* (2012): (1) prepubescent: predominant characteristics are homogeneous oocytes without expanded stroma or vitellogenic follicles; (2) pubescent: ovaries with little expansion, and previtellogenic follicles in different developmental stages; and (3) mature: presence of vascularized expanded stroma, previtellogenic and vitellogenic follicles. In

regards to males, the determination of maturity stages followed classification from Wibbels *et al.* (1990) and Otsuka *et al.* (2008): (1) prepubescent: seminiferous tubules with small diameter, presence of spermatogonias, and well-developed interstitial connective tissue; (2) pubescent: seminiferous tubules with spermatogonias and spermatocytes, expansion in seminiferous tubules and reduction in interstitial connective tissue; and (3) mature: spermatogenesis approaches maximum, larger seminiferous tubules contain more layers of cells in the germinal epithelium, and abundant sperm in the lumen. Through microscopic analysis we confirmed the sex of some individuals and classified their maturation stages based on characteristics of germinative cells present in their gonads. We used the χ^2 test to evaluate for statistically significant differences between number of sampled females and males. The significance level (alpha) was 5% ($p < 0.05$) and statistical analyzes were performed with Statistica software, version 7.0.

During the study period, 3,337 stranded green turtles were recorded (range = 23-132 cm CCL), and the macroscopic determination of sex was possible in 1,130 individuals considering their state of decomposition. Seventy-eight percent of the turtles were females ($N = 877$), and 22% were males ($N = 253$) ($\chi^2 = 344.54$; g.l. = 1; $p < 0.05$) (sex ratio = 3.46F:1M). These results are consistent with a study conducted in Rio Grande do Sul - Brazil that also found more females than males (2.8F:1M; Duarte *et al.* 2011). Research has indicated higher rates of females being produced from different nesting areas over the years (Wibbels 2003; King *et al.* 2013; Marcovaldi *et al.* 2014). Climate change may be linked to these results, and an increase of 2 $^{\circ}$ C in some nesting areas is predicted to result in fully feminized nests, while 3 $^{\circ}$ C increase

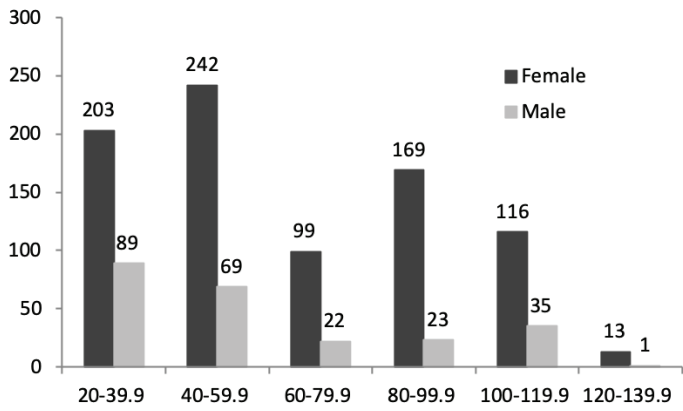


Figure 2. Absolute frequency of female and male green sea turtles stranded in the Potiguar Basin/RN-CE between 2011 and 2015 according to their size range.

would be considered a lethal incubation temperature (Hawkes *et al.* 2007). Studies conducted in the NE Caribbean suggest that in the last several decades only 15.5% of green sea turtles produced each year were male, and global warming may exacerbate this trend (Laloë *et al.* 2016).

Some individuals were found with broken carapaces, and we could not accurately classify their size. Therefore, a total of 1,130 green sea turtles were examined macroscopically and 1,081 were classified into size bins. The number of females was higher than males in all size categories (Fig. 2): 20-39.9 (N = 292; 69.5% females), 40-59.9 (N = 311; 77.8% females), 60-79.9 (N = 121; 81.8% females), 80-99.9 (N = 192; 88% females), 100-119.9 (N = 151; 76.8% females), and 120-139.9 (N = 14; 92.9% females). Data obtained between 1 January 2010 and 31 December 2012 in Potiguar Basin revealed 1,142 stranded green turtles; 71% were under 60 cm CCL, and thus were classified as juveniles (Gavilan-Leandro *et al.* 2016). Another study carried out during 2007-2012 in Anchieta - Espírito Santo state, Brazil, reported that 97% of 1,094 stranded green turtles were juveniles (Berrêdo *et al.* 2013). In southern Brazil, green sea turtles begin their coastal development period at around 30-40 cm carapace length (Lenz *et al.* 2017). Our findings suggest

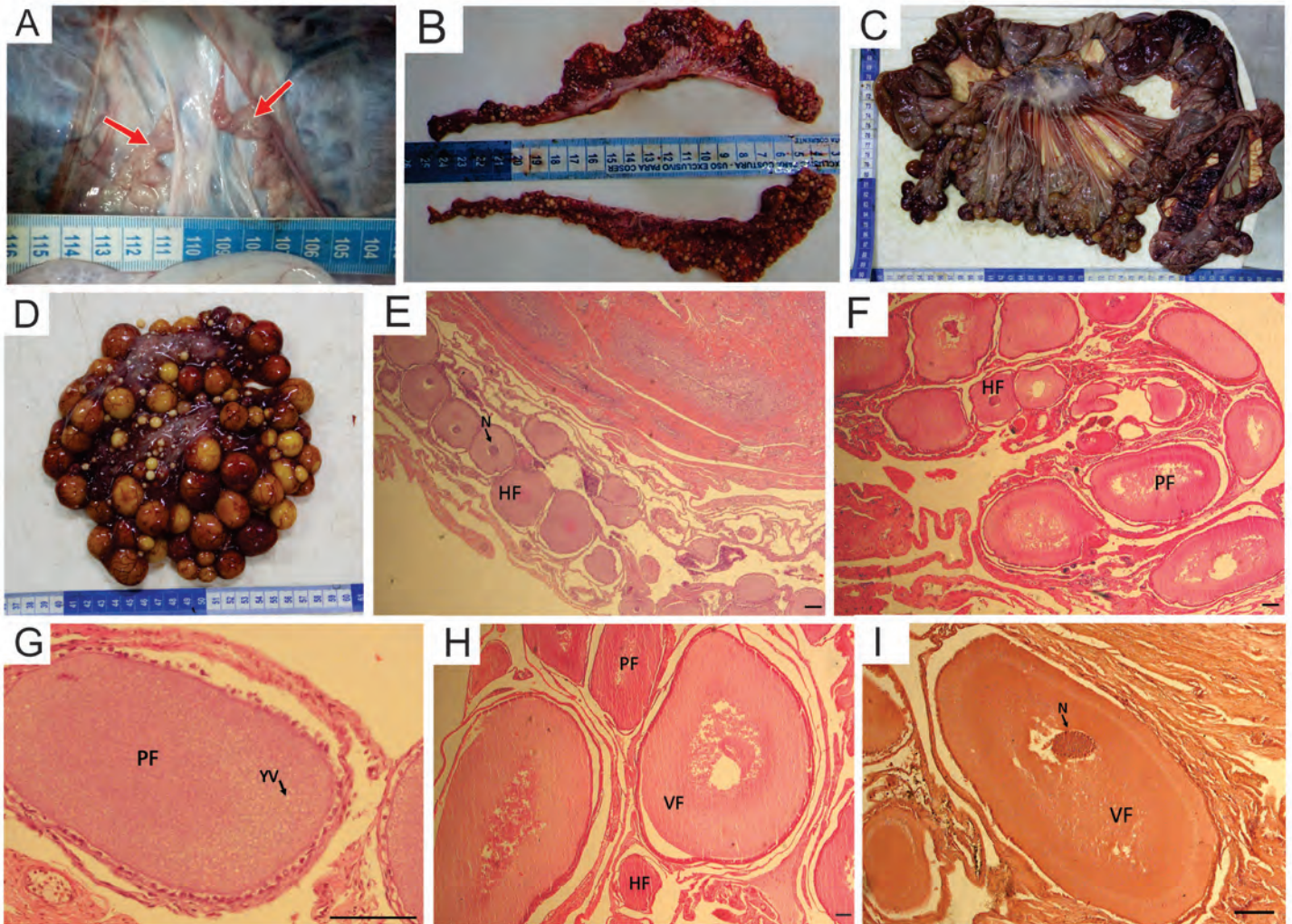


Figure 3. Maturation stages of female green sea turtles: (A) Prepubescent (red arrow: ovaries); (B) Pubescent; (C and D) Mature. Histological section of ovaries: (E) Prepubescent (CCL = 42 cm); (F and G) Pubescent (CCL = 62.1 cm and 64 cm, respectively); (H and I) Mature (CCL = 107 cm and 89 cm, respectively); N: nuclei; HF: homogeneous follicles; PF: previtellogenic follicles; YV: yolk vesicles, and VF: vitellogenic follicles. Bar = 100 μ m.

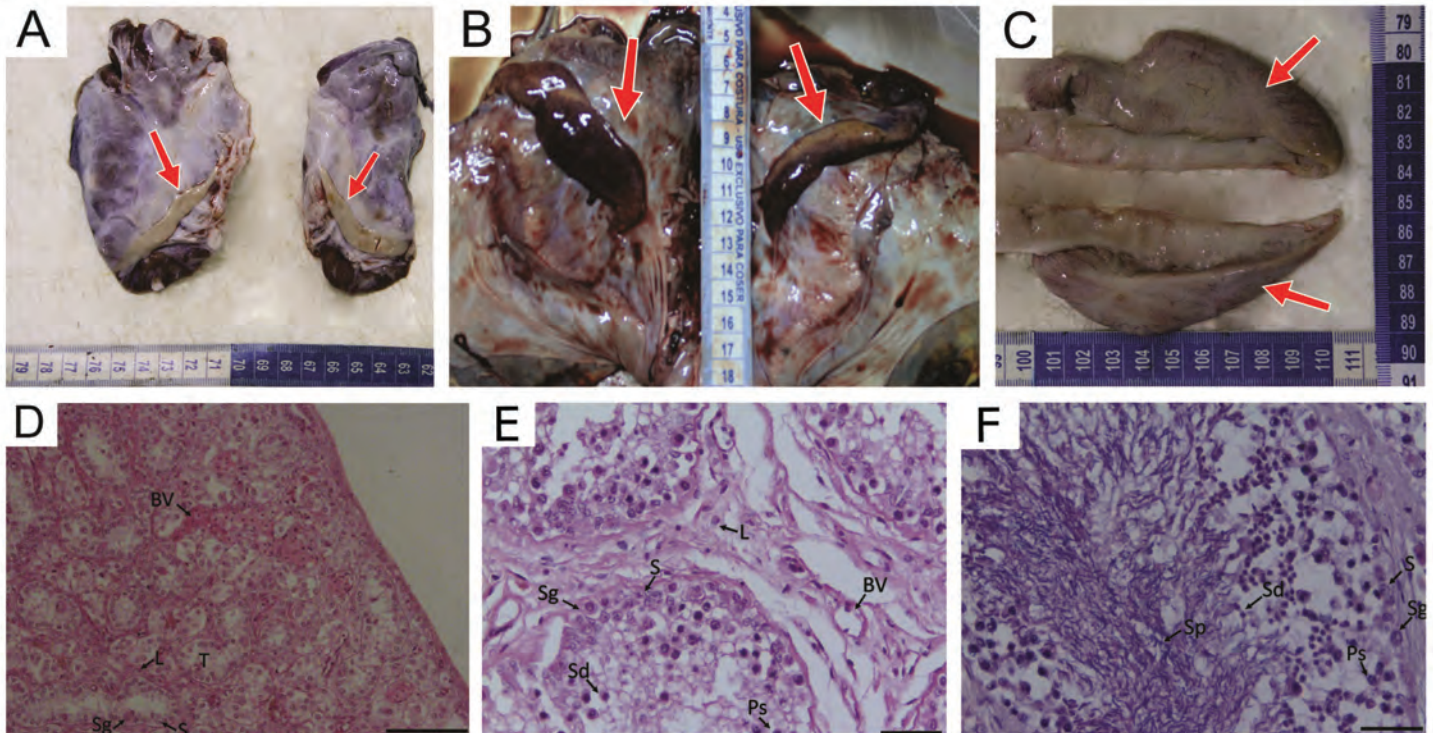


Figure 4. Maturation stages of male green sea turtles: (A) Prepubescent; (B) Pubescent; (C) Mature. Red arrows: testes. Histological section of testes: (D) Prepubescent (CCL = 32.3 cm) (Bar = 100 μ m); (E) Pubescent (CCL = 93 cm) (Bar = 20 μ m); (F) Mature (CCL = 105 cm) (Bar = 20 μ m). T: seminiferous tubule; S: Sertoli cell; L: Leydig cell; BV: blood vessels; Sg: spermatogonia; Ps: primary spermatocyte; Sd: spermatid; Sp: sperm.

a strong probability that juvenile green turtles use Potiguar Basin and its adjacent areas as their feeding ground.

Macroscopic analysis of gonads revealed differences in appearance for females at different stages of maturity. Gonads of females in the prepubescent stage were pinkish in color with low vascularization, grainy texture, and smaller than mature gonads (Fig. 3A). Pubescent female gonads were pink in color, irregularly shaped, elongate, fusiform in outline, and more vascularized and granular than prepubescent ones (Fig. 3B). In mature ovaries, we observed in numerous round intensely yellow oocytes (Figs. 3C and 3D). Through histological examination, we observed that prepubescent females had ovaries with small follicles and homogeneous cytoplasm, without expanded stroma and reserve material (Fig. 3E), as described by previous studies (Rosa 2009; Pérez *et al.* 2010). Pubescent ovaries presented previtellogenic follicles with increased cytoplasm containing some lipid vesicles indicating the reserve material production, and could be characterized as initial vitellogenesis (Figs. 3F and 3G); these features were also found by Pérez *et al.* (2010). The presence of lipids in the oocyte cytoplasm in previtellogenic stages has been described in other reptiles and marine turtles, indicating that at this stage the oocyte grows rapidly due to the transcriptional activity of chromosomes and the strong metabolism associated with lipid synthesis (Guraya 1989; Pérez-Bermúdez *et al.* 2012). In mature ovaries, follicles were larger in diameter than in previous stages, and yolk platelets, vitellogenic follicles and follicles in different developmental stages were present (Figs. 3H and 3I). Pérez-Bermúdez *et al.* (2012) also described these structures, indicating that in vitellogenesis the yolk platelets progressively increase in diameter occupying all the cytoplasm. Several sizes of vitellogenic and previtellogenic follicles coexist in

adult reproduction, which may be indicative of different clutches produced during a single nesting season or groups of follicles that will initiate vitellogenesis before the next breeding season.

In males, the prepubescent gonads were white in color, smooth in texture, and presented low vascularization (Fig. 4A). The pubescent testes were more vascularized and enlarged than prepubescent ones (Fig. 4B). The mature gonads were highly vascularized and attached to the body wall by its flat dorsal surface (Fig. 4C). Histologically, most males were classified as prepubescent and presented seminiferous tubules with smaller diameter than mature testes, and seminiferous epithelium composed of Sertoli cells and Leydig cells within interstitial connective tissue (Fig. 4D); these characteristics were also described by Otsuka *et al.* (2008). Pubescent testes had expanded seminiferous tubules and layers of cells composed by spermatogonia, primary spermatocytes, spermatids and Sertoli cells, blood vessels, and Leydig cells in interstitial connective tissue (Fig. 4E). This is similar to a study conducted by Pérez *et al.* (2010), that reported that primary and secondary spermatocytes, spermatids, and sperm were scarce in the seminiferous tubules. Finally, the only individual classified as a mature male (CCL = 105 cm) had sperm within the seminiferous tubular lumen, larger diameter than previous stages, and germinative epithelium with several layers of different types of cells (Fig. 4F). According to Wibbels *et al.* (1990), these features indicate that a male has already developed the entire spermatogenic cycle.

We verified that macroscopic analysis was not appropriate to determine sex of prepubescent females and males because of the gross similarity in their gonads, and we suggest using the microscopic analysis as described by Wibbels (2003) and Ceriani & Wyneken (2008). During the years before sea turtles reaches puberty,

Stage of gonadal maturation	Sex	Number	Curved carapace length (cm)			
			Minimum	Maximum	Mean \pm SD	Median (Q1; Q3)
Prepubescent	F	35	29	59	37.4 \pm 7.1	36 (33; 40)
	M	17	27	60.5	39.5 \pm 10.7	35 (31.5; 48.4)
Pubescent	F	12	61	95	77.5 \pm 12.6	80 (64.25; 90.5)
	M	7	66	114	89.9 \pm 17.8	93 (69; 104.5)
Mature	F	6	89	107	100.8 \pm 6.8	103 (95; 106.25)
	M	1	-	-	105*	105*

Table 1. Curved carapace length of green sea turtles stranded in the Potiguar Basin/Rio Grande do Norte-Ceará, Brazil (2011-2015) according to their stages of gonadal maturation. F: Female; M: Male; SD: Standard deviation; Q1: 25th percentile; Q3: 75th percentile. *Absolute value.

gonads increase in size, and their morphological differences become increasingly more visible (Miller & Limpus 2003). In the puberty stage, hormonal changes increase the size and structure of ovaries and oviduct; and during spermatogenesis, testosterone influences the differentiation from Sertoli cells into seminiferous tubules (Hamann *et al.* 2003). A previous study on Brazilian juvenile green sea turtles at 27.5-68 cm, revealed that gonads of both sexes can present mixed textures and the same shape: 80% of females had ovaries with grainy texture, while 71% of males presented testes smooth in texture; 24 gonads were considered attached to the body wall (21 from males and 3 from females), and 72 were characterized as non-attached (49 ovaries and 23 testes; Rosa 2009). In our research, microscopic analysis was useful to confirm the sex of five males (27, 29, 31, 85 and 93 cm CCL) and two females (61 and 65 cm CCL), which could not be classified according to macroscopic characteristics such as texture or adherence.

In Brazil, minimum size of reproductive maturation in green sea turtles is based on the smallest documented carapace length for nesting sea turtle females in the largest nesting area within close proximity (CCL = 90 cm for *C. mydas*; Almeida *et al.* 2011). Our study considered CCL associated with stages of gonadal maturation (Table 1). We observed one female under 90 cm CCL (89 cm CCL) with histological characteristics described for an adult, and three other individuals (CCL > 90 cm) that were considered pubescent according to histological description of their ovaries. Research carried out in Paraná state, Brazil revealed that the gonadal development of green turtles was concomitant with their carapace growth (Rosa 2009). However, studies conducted in Cuba concluded that the follicular phases found in ovaries of hawksbill turtles were statistically independent of their carapace length (Pérez-Bermúdez *et al.* 2012). With regards to males, we classified one individual as pubescent (CCL = 114 cm) based on the absence of sperm within seminiferous tubules; whereas the only individual classified as mature was smaller (CCL = 105 cm). Pubescent hawksbill turtles demonstrated signs of spermatogenic activity upon histological analysis but lacked a fully developed penis, indicating asynchrony between testicular and penile developments; and another individual was considered adult male according to phenotypic characteristics (carapace length and penis size) but without histological structures consistent with sexual maturity (Pérez *et al.* 2010). As stated by Otsuka *et al.* (2008), the straight carapace length is not an adequate indicator of maturity, but histological analysis is more definitive.

It is well known that age and size of marine turtles can vary due

to environmental temperatures and food availability in foraging grounds, and populations with high genetic variation are more resistant to environment changes that can interfere in development (Limpus & Nicholls 2000; Joseph & Shaw 2011). There is a paucity of research on maturation stages associated with carapace length, perhaps due to the logistic challenges associated with finding and capturing males for research. In the last decade, the required implementation of the Beach Monitoring Program in Brazil has led to an increase in research on the biology of marine vertebrates, especially sea turtles. Our results indicate that carapace length is not appropriate to identify maturation stage, and histological examination should be used, when possible, as a suitable technique for this purpose. This study also provided sex ratio data for green turtles stranded in Potiguar Basin, and important information about gonad morphology in different phases of sexual development.

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Northernmost Bycatch Record of an Olive Ridley Turtle (*Lepidochelys olivacea*) in the Pacific Coast of Japan

Takuya Fukuoka, Chihiro Kinoshita & Katsufumi Sato

Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba, 277-8564, Japan
(E-mail: t.fukuoka2@gmail.com; chichiro.kinoshita@gmail.com; katsu@aori.u-tokyo.ac.jp)

The olive ridley turtle, *Lepidochelys olivacea*, is considered the most abundant sea turtle species in the world (Márquez 1990; Pritchard 1997) and is listed on the IUCN Red List as Vulnerable (Abreu-Grobois & Plotkin 2008). This species has a circumtropical distribution, with nesting occurring throughout tropical waters (Plotkin 2003; Abreu-Grobois & Plotkin 2008). Although the main nesting grounds where arribada nesting occurs are distributed throughout the eastern Pacific Ocean from Mexico to Costa Rica and the northeast coast of India (Márquez 1990; Pritchard 1997), several nesting grounds have been reported in the northwest Pacific Ocean from Indonesia to Taiwan (Suganuma 1994; Abreu-Grobois & Plotkin 2008). During foraging periods, juvenile and adult olive ridley turtles reside in a wide range of tropical and sub-tropical waters in neritic and oceanic habitats (Márquez 1990; Plotkin 2003). In the eastern Pacific Ocean, there are two stranding records from extremely high latitudes (up to 59.5 °N), but these were carcasses (Hodge & Wing 2000).

There are no records of nesting olive ridley turtles in the Japanese archipelago, which is mainly situated in temperate water. However, >50 bycatch and stranding records of olive ridley turtles exist from 1957-2015 (Nishimura *et al.* 1972; Kuroyanagi & Masuda 1992; Suganuma 1994; Kamezaki *et al.* 1995; Kuramoto & Ishii 2003; Tsutsumi & Hashimoto 2003; Kino & Kawazu 2014; Shimura *et al.* 2017; Fig. 1, Table 1). Suganuma (1994) noted that the majority of the bycatch occurred during summer and autumn when the water temperature is high (20-30 °C; Table 1). In contrast, stranding records were concentrated during winter (Table 1). These results suggested that olive ridley turtles utilize Japanese coastal waters for seasonal foraging and are unable to tolerate the low water temperatures during winter (Suganuma 1994). In the Pacific coast of northeastern Japan (>35 °N), which is the cool-temperate region with lower water temperature (4- 20 °C), there is no evidence that olive ridleys occurred previously, with the exception of one bycaught turtle from oceanic waters recorded in area 545 of the fishing grounds (42.50-43.00 °N, 152.50-153.00 °E) (Suganuma 1994).

Olive ridley turtles are omnivores that feed on gelatinous prey, benthic animals and macroalgae (Bjørndal 1997; Peavey *et al.* 2017). In Japan, benthic animals such as crustaceans, snails, bivalves and sea urchins were found in the feces of two olive ridley turtles stranded in Ise Bay (Kuroyanagi & Masuda 1992; Fig. 1). However, no other diet information has been reported in Japan until this study. Here, we describe the fecal contents of the first olive ridley bycatch record from neritic waters of the cool-temperate Sanriku Coast, northeastern Japan.

The Sanriku Coast is one of the most productive areas in Japan because of the mixing of waters from the Tsugaru Warm Current, the cold nutrient-rich Oyashio Current, and a branch of the warm Kuroshio Current (Hanawa & Mitsudera 1987; Sugimoto & Tameishi 1992; Fig. 1). Since 2005, the incidental capture of sea turtles by commercial set nets has been surveyed in this area, and

456 loggerhead (*Caretta caretta*), 145 green (*Chelonia mydas*), 10 leatherback (*Dermochelys coriacea*), six black (*C. mydas agassizii*), and one hawksbill (*Eretmochelys imbricata*) turtles were captured during June to November from 2005 to 2019. This result indicated that the Sanriku Coast is a summer-restricted habitat for sea turtles (Fukuoka *et al.* 2015; Narazaki *et al.* 2015; Sato *et al.* 2016).

On 21 July 2016, one olive ridley turtle was incidentally captured by a set net at Funakoshi Bay, Iwate prefecture, Japan (39.40 °N, 141.97 °E; Fig. 1). The turtle was identified as *Lepidochelys olivacea* based on diagnostic features including the presence of six pairs of lateral scutes, olive-grey scute and skin color, circular shape of the carapace, and two pairs of prefrontal scales (Figs. 2a, 2b; Márquez 1990). The water temperature of Funakoshi Bay at 3m depth was 16.9 °C when the turtle was captured.

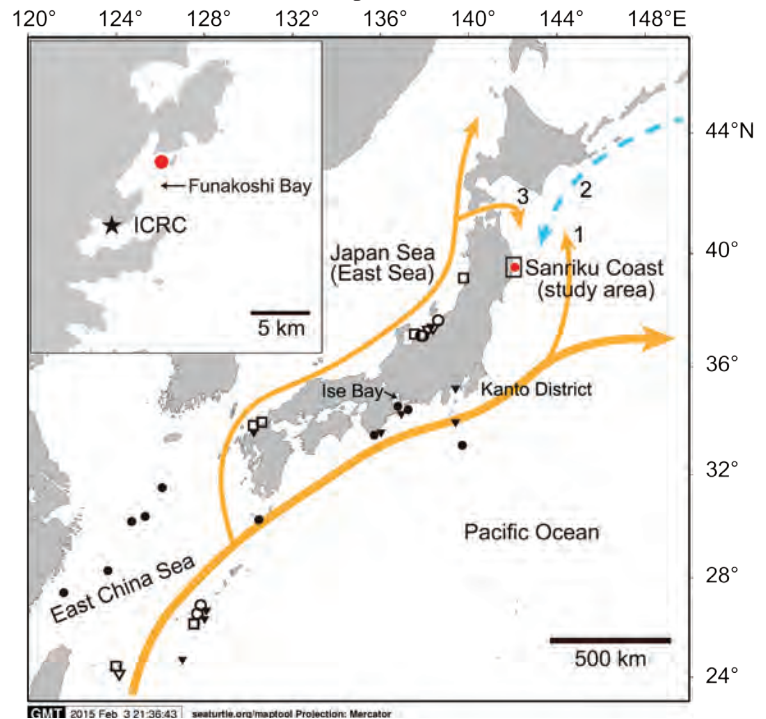


Figure 1. Study site on the Japanese archipelago and locations of olive ridley bycatch (filled markers) and stranding (open markers) records. The records of unknown locations and unknown capture types were not shown. Difference in marker shape means captured alive (circles), dead (squares), and unknown (triangles), respectively. The red circle indicates the capture location in this study. Orange solid and blue dashed lines illustrate warm and cold currents, respectively. Numbered currents show 1: branch of the warm Kuroshio Current, 2: cold Oyashio Current, and 3: Tsugaru Warm Current. Inset is a magnification of the study site (open rectangle). A star marks the International Coastal Research Center (ICRC).

Area	Location	Lat/Long	Date (mm/dd/yyyy)	Capture type	Dead/ Alive	Size (cm)	References
Pacific Ocean	Area 545 of the fishing ground	42.5-43.0 °N, 152.5-153.0 °E	7/11/1981	Bycatch	UN	31 (U)	Suganuma 1994
	Otsuki, Kochi Pref.	32.7-32.9 °N, 132.6-132.9 °E	May 1971	UN	UN	UN	Suganuma 1994
	Kushimoto, Wakayama Pref.	33.4-33.5 °N, 135.6-135.9 °E	10/11/1971	Bycatch	Alive	58 (U)	Nishimura et al. 1972
	Kushimoto, Wakayama Pref.	33.4-33.5 °N, 135.6-135.9 °E	11/5/1971	Bycatch	Alive	55 (U)	Nishimura et al. 1972
	Off the Shionomisaki, Wakayama Pref.	UN	6/16/1972	Bycatch	UN	50.0 (S)	Suganuma 1994
	Daio, Mie Pref.	34.3 °N, 136.9 °E	8/9/1972	Bycatch	UN	55 (C)	Suganuma 1994
	Daio, Mie Pref.	34.3 °N, 136.9 °E	10/13/1972	Bycatch	UN	UN	Suganuma 1994
	Gamouda, Tokushima Pref.	33.8 °N, 134.7 °N	7/30/1974	UN	UN	56.3 (U)	Suganuma 1994
	Off the Ishigaki Is, Okinawa Pref.	24.3 °N, 127.0 °E	6/11/1975	Bycatch	UN	40.4 (U)	Suganuma 1994
	Off the Kuzura, Shizuoka Pref.	35.0 °N, 138.9 °E	7/29/1978	UN	UN	UN	Suganuma 1994
	Oiso, Kanagawa Pref.	35.3 °N, 139.3 °E	1983	Bycatch	UN	UN	Suganuma 1994
	Off the Izu Islands, Tokyo Pref.	33.1-34.7 °N, 138.9-139.7 °E	1983	Bycatch	UN	62.5 (S)	Suganuma 1994
Pacific coast	Off the Izu Islands, Tokyo Pref.	33.1-34.7 °N, 138.9-139.7 °E	1983	Bycatch	UN	UN	Suganuma 1994
	Ago, Mie Pref.	34.2-34.4 °N, 136.7-136.9 °E	7/27/1983	UN	UN	UN	Suganuma 1994
	Off the Kushimoto, Wakayama Pref.	UN	11/5/1987	Bycatch	UN	UN	Suganuma 1994
	Kin, Okinawa Pref.	26.4 °N, 127.8- 128.0 °E	1988	Bycatch	UN	UN	Suganuma 1994
	Off the Irigo, Aichi Pref.	34.5 °N, 137.0 °E	10/20/1988	Bycatch	Alive	UN	Kuroyanagi & Masuda 1992
	Shionomisaki, Wakayama Pref.	33.4 °N, 135.8 °E	9/30/1989	Sighting	UN	UN	Suganuma 1994
	Nachi-katsuura, Wakayama Pref.	33.5-33.7 °N, 135.9-136.0 °E	10/23/1989	Bycatch	UN	64.8 (S)	Suganuma 1994
	Off the Kamishima, Mie Pref.	34.5 °N, 137.0 °E	11/7/1991	Bycatch	Alive	56.8 (S)	Kuroyanagi & Masuda 1992
	Off the Hachijo Is, Tokyo Pref.	32.8 °N, 139.6 °E	10/12/2002	Bycatch	Alive	53.5 (S)	Tsustumi & Hashimoto 2003
	Funakoshi Bay, Iwate Pref.	39.40 °N, 141.97 °E	7/21/2016	Bycatch	Alive	50.6 (S)	This study
	Shikanoshima Is, Fukuoka Pref.	33.7 °N, 130.3 °E	UN	Stranding	Dead	UN	Kuramoto & Ishii 2003
	Shikanoshima Is, Fukuoka Pref.	33.7 °N, 130.3 °E	Aug 1957	Bycatch	UN	UN	Nishimura et al. 1972
	Teradomari, Niigata Pref.	37.6 °N, 138.8 °E	1/12/1964	Stranding	UN	62 (U)	Suganuma 1994
Japan Sea	Nishiyama, Niigata Pref.	37.4-37.5 °N, 138.6-138.7 °E	12/12/1965	Stranding	UN	60 (U)	Suganuma 1994
	Sakata, Yamagata Pref.	38.9 °N, 139.8 °E	1/3/1968	Stranding	Dead	21 (C)	Nishimura et al. 1972
	Kashiwazaki, Niigata Pref.	37.3-37.4 °N, 138.4-138.6 °E	1/26/1968	UN	UN	15 (U)	Kino & Kawazu 2014
	Kashiwazaki, Niigata Pref.	37.3-37.4 °N, 138.4-138.6 °E	12/14/1972	Stranding	Alive	58.7 (U)	Suganuma 1994

Table 1. Olive ridley turtle occurrences in Japan. UN = unknown. For size, (S) is straight carapace length, (C) is curved carapace length, and (U) is unknown if straight or curved carapace length.

Area	Location	Lat/Long	Date (mm/dd/yyyy)	Capture type	Dead/ Alive	Size (cm)	References
Japan Sea	Niigata, Niigata Pref.	37.7-38.0 °N, 138.8-139.2 °E	1/25/1974	Stranding	Alive	55.5 (U)	Suganuma 1994
	Kakizaki, Niigata Pref.	37.2-37.3 °N, 138.3-138.4 °E	1/27/1976	Stranding	Alive	54 (U)	Suganuma 1994
	Oga, Akita Pref.	39.9-40.1 °N, 139.7-140.0 °E	2/7/1979	UN	UN	66 (C)	Suganuma 1994
	Joetsu, Niigata Pref.	37.1-37.2 °N, 138.1-138.3 °E	Jan 1987	Stranding	Dead	65 (U)	Suganuma 1994
	Tsuyazaki, Fukuoka Pref.	33.8 °N, 130.5 °E	12/4/1993	Stranding	Dead	UN	Kuramoto & Ishii 2003
	Tsuyazaki, Fukuoka Pref.	33.8 °N, 130.5 °E	12/11/1993	Stranding	Dead	66 (U)	Kuramoto & Ishii 2003
	Nishikanbara, Niigata Pref.	UN	12/21/1995	UN	UN	59.7 (U)	Kino & Kawazu 2014
	Shiunji, Niigata Pref.	UN	1/25/1997	UN	UN	60.7 (U)	Kino & Kawazu 2014
	Ryotsu, Niigata Pref.	UN	2/2/1997	UN	UN	55.7 (U)	Kino & Kawazu 2014
East China Sea	East China Sea	UN	1958 summer	Bycatch	Alive	UN	Nishimura et al. 1972
	East China Sea	UN	1958 autumn	Bycatch	Alive	UN	Nishimura et al. 1972
	Off the Amami-Oshima, Kagoshima Pref.	28.3-28.4 °N, 129.3-129.4 °E	9/26/1967	Captured	Alive	47 (U)	Nishimura et al. 1972
	East China Sea	30.3 °N, 125.2 °E	8/28/1969	Bycatch	Alive	47 (U)	Nishimura et al. 1972
	East China Sea	30.3 °N, 124.8 °E	9/8/1969	Bycatch	Alive	48 (U)	Nishimura et al. 1972
	East China Sea	27.8 °N, 122.3 °E	9/8/1971	Bycatch	Alive	55.2 (U)	Nishimura et al. 1972
	East China Sea	28.2 °N, 124.3 °E	12/8/1971	Bycatch	Alive	58 (U)	Nishimura et al. 1972
	East China Sea	31.5 °N, 126.2 °E	12/11/1971	Bycatch	Alive	53 (U)	Nishimura et al. 1972
	East China Sea	UN	10/24/1977	Bycatch	UN	61 (U)	Suganuma 1994
	Off the Motobu, Okinawa Pref.	26.6-26.7 °N, 127.8-127.9 °E	1988	Bycatch	UN	UN	Suganuma 1994
	Zamami Is, Okinawa Pref.	26.2 °N, 127.3 °E	Nov 1994	Stranding	Dead	17.0 (S)	Kamezaki et al. 1995
	Yakushima Is, Kagoshima Pref.	30.4 °N, 130.4 °E	11/2/1994	Bycatch	Alive	48.6 (S)	Kamezaki et al. 1995
	Taketomi Is, Okinawa Pref.	24.3 °N, 124.1 °E	12/28/1994	Stranding	Dead	34.9 (S)	Kamezaki et al. 1995
	Kuroshima Is, Okinawa Pref.	24.2 °N, 124.0 °E	12/26/2001	Stranding	UN	57.4 (S)	Kino & Kawazu 2014
Nago, Okinawa Pref.	26.7 °N, 128.0 °E	11/4/2013	Stranding	Alive	13.5 (S)	Kino & Kawazu 2014	
Onna, Okinawa Pref.	26.5 °N, 127.9 °E	1/13/2015	Stranding	Alive	18.0 (S)	Shimura et al. 2017	

Table 1. Continued. Olive ridley turtle occurrences in Japan. UN = unknown. For size, (S) is straight carapace length, (C) is curved carapace length, and (U) is unknown if straight or curved carapace length.

The olive ridley turtle was found alive but emaciated and in captivity it always floated on the water surface in the tank (Fig. 2c). In addition, the turtle had lacerations on its plastron; unfortunately, we could not identify their cause (Fig. 2d). Therefore, it was transferred to an indoor tank (1.5 × 1.2 × 0.6 m) at the International Coastal Research Center (ICRC, Fig. 1), Atmosphere and Ocean Research Institute, the University of Tokyo, Otsuchi town, Iwate prefecture, Japan. Straight carapace length (SCL) and straight carapace width (SCW) were measured to the nearest 0.1 cm with calipers. Curved carapace length (CCL) and curved carapace width (CCW) were measured to the nearest 0.1 cm with a flexible tape measure. Body mass (BM) was measured to the nearest 0.5 kg with a hanging scale. When the olive ridley turtle was captured, SCL,

CCL, SCW, CCW and BM were 50.6 cm, 53.0 cm, 46.3 cm, 56.1 cm and 16.0 kg, respectively. A sexually mature olive ridley turtle has been estimated to have an SCL of 60 cm approximately, typically a minimum of 52.5 cm and maximum of 75 cm (Hirth 1980; Zug *et al.* 2006). Hence, the olive ridley turtle captured at the Sanriku Coast was considered to be a large juvenile. This is a common size for previous records of olive ridley turtles in other parts of Japan (40-60 cm SCL, Table 1). Two *Planes* crabs, *Planes major* (male and female), which are frequently attached to loggerhead and olive ridley turtles (Frick & Pfaller 2013; Pfaller *et al.* 2014) were found on the tail of the olive ridley turtle when the turtle was captured.

For the first week after capture, the water temperature of the tank was set at 25 °C using a thermo-controller (REI-SEA TC-101,

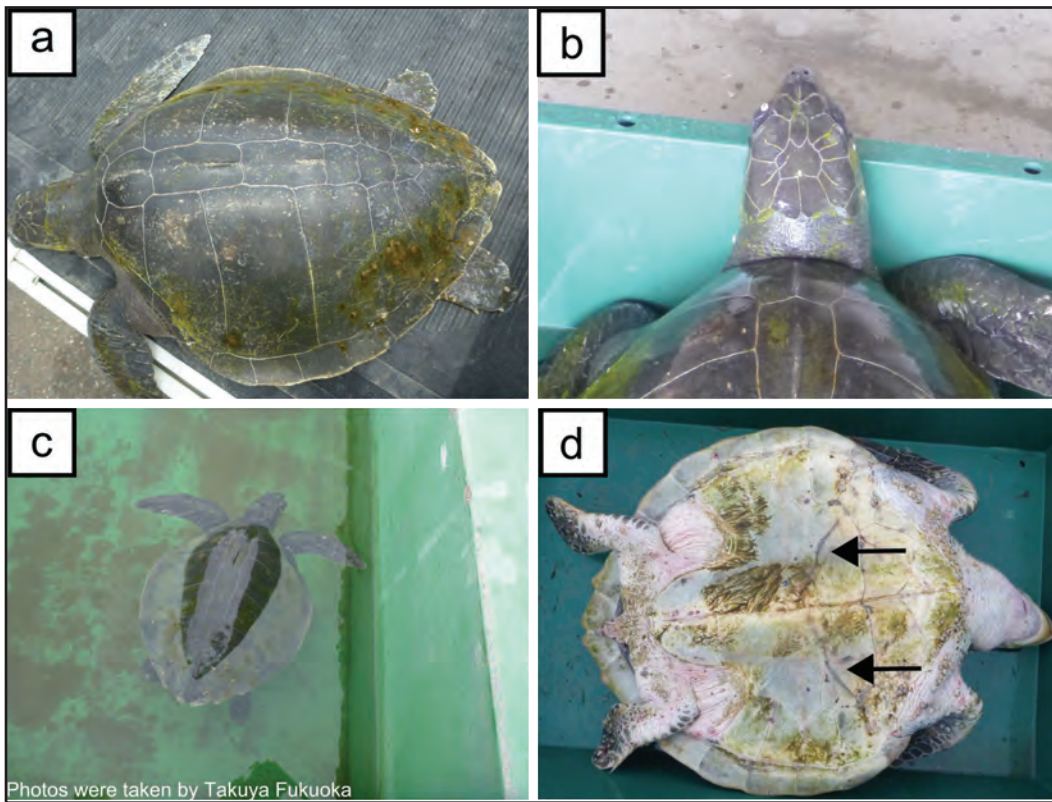


Figure 2. An olive ridley turtle captured by a commercial set net along the Sanriku Coast, Japan. (a) Carapace, and (b) head of the turtle. Six pairs of lateral scutes and two pairs of prefrontal scales exist on the carapace and head, respectively. (c) The turtle floating on the water surface. (d) Lacerations (two black arrows) on the plastron.

Iwaki Co, Tokyo, Japan). Afterwards, the turtle was transferred to an outdoor tank (3.6 × 1.5 × 1.0 m) at the same institute. The outdoor tank water temperature was maintained >25 °C (approximate range: 25-30 °C) due to the warm air temperatures heating the water. After being in water >25 °C for a week, the turtle became active and started diving and eating fish fillets, squid, and mussels. However, the pump system of ICRC failed in September, and the turtle died on 20 September 2016 when the water temperature dropped rapidly <20 °C (approximately range: 15-20 °C) because of low air temperatures around 15 °C.

During the first week of the captive period, we sampled and analyzed feces. The tank that housed the olive ridley turtle was checked daily, and feces found in the tank were collected using a net. To collect as much fecal matter as possible, we did not flush the water in the tank during the feces sampling period. After the turtle was transferred to the outdoor tank, we stopped the sampling because some algae and marine debris were contaminating the tank water through the seawater pump system. Following Fukuoka *et al.* (2016), items within the fecal samples were classified as diet items, natural debris, and artificial debris. Diet items were identified to the lowest taxonomic level possible by visual examination. Wet mass of each sample was weighed to 0.1 g using a digital scale. Of a total of 62.9 g of feces sample, 52.7 g of Salpidae was present, representing 83.8% of the total mass, and 6.7 g of artificial debris was identified, representing 10.7% of the total mass (Table 2). Brown, green and red algae were also found in the feces but only in small amounts (<2 g).

The fecal analysis indicated that prior to capture, the olive ridley turtle mainly fed on floating materials such as salps. Salpidae are commonly found in the gut contents of olive ridley turtles in the Pacific Ocean (Bjorndal 1997; Wedemeyer-Strombel *et al.* 2015), although this was not found in a previous study of two olive ridley turtles from Ise Bay, along the southern coast of Japan (Kuroyanagi & Masuda 1992). Salps are present and available in both neritic and

oceanic waters (Henschke *et al.* 2016). *Planes* crabs are typically found drifting in pelagic/oceanic waters (Frick & Pfaller 2013; Pfaller *et al.* 2014). Because *Planes* crabs were found on the captured olive ridley, and salps were determined to be part of the olive ridley turtle's diet, we suggest that this turtle utilized oceanic waters before migrating to the Sanriku Coast. Because a branch of the Kuroshio Current flows northwards off the Sanriku Coast (Kawai 1972), this olive ridley turtle may have travelled north following this current.

A previous study reported that artificial debris, which was the second most abundant item in this study, was frequently ingested by sea turtles in the Pacific Ocean (Wedemeyer-Strombel *et al.* 2015). According to gut content analyses, loggerhead and green turtles migrating to the Sanriku Coast display a high frequency of occurrence of debris ingestion (>80 %; Fukuoka *et al.* 2016). Artificial debris ingestion by the olive ridley in this study confirmed that three sea turtle species along the Sanriku Coast may ingest debris. A recent study addressed sub-lethal effects of debris ingestion such as body condition and transfer of toxic compounds (Clukey *et al.* 2018). Hence, we consider it necessary to conduct such a study along the Sanriku Coast where sea turtles ingest artificial debris.

The present study is the most recent and northernmost record of an olive ridley turtle along the Pacific coast of Japan, approximately 500 km north from the previous record in Kanto district (Fig. 1, Table 1). The Sanriku Coast is highly productive; however, the period when water temperatures exceed 20 °C, which is considered a suitable temperature for olive ridley turtles, is shorter than two months annually (Sato *et al.* 2007). When the turtle was captured in water temperatures of 16.9 °C, it was emaciated. The turtle became active and started diving when the water temperature was maintained >25 °C, suggesting that this individual experienced hypothermia because of low water temperatures when it was captured. Therefore, it is inferred that the northern limit of seasonal foraging areas of olive ridley turtles is the southern coastal waters of Japan, and along

Category	wet mass (g)	% of the total mass
Animalia		
Chordata		
Thaliacea		
Salpida		
Salpidae	52.7	83.8
Plantae		
Phycophyta		
Phaeophyceae		
Fucales		
Sargassaceae		
Sargassum	1.5	2.4
Florideophyceae	0.2	0.3
Ulvophyceae	0.2	0.3
Unknown	0.1	0.2
Other material		
Bird feathers	1.3	2
Wood/Leaves	0.2	0.3
Artificial debris	6.7	10.7
Total	62.9	100

Table 2. Wet mass (grams and % of total mass) of different taxa and other materials in the sample of *Lepidochelys olivacea* feces.

the Sanriku Coast is considered unfavorable habitat for the olive ridley turtle even during the summer.

It is reported that water temperatures off the Sanriku Coast have risen 0.68 °C during the last 100 years (The Japan Meteorological Agency 2017). In July 2016 when this olive ridley turtle was captured, water temperature off the Sanriku Coast was 2-4 °C warmer than the average water temperature from 1986 to 2016 (The Japan Meteorological Agency 2016). We cannot associate the relationship between this northernmost bycatch of an olive ridley turtle and rising ocean temperatures because it is only one record. However, it has been reported that many marine mammal species display poleward distribution shifts in their geographical ranges, in relation to rising water temperatures (Tasker 2008; Poloczanska *et al.* 2013; Kleisner *et al.* 2017). Moreover, some studies predicted that sea turtles also change their habitat accompanied by rising water temperature (Chaloupka *et al.* 2008; Witt *et al.* 2010; Hazen *et al.* 2012). Further monitoring, and continued bycatch surveys in Japan in the northern limits of marginal habitat for olive ridley turtles, is important to examine whether this species is shifting their distribution as water temperatures increase around the globe.

Acknowledgements. The present study was incidentally conducted during a tag and release program where loggerhead and green sea turtles were caught by commercial set nets as bycatch in the Sanriku Coast and then turned in by fisherman to researchers. The program was performed in accordance with the guidelines of the Animal Ethic Committee of the University of Tokyo, and the protocol of this study was approved by the committee (P 16-5). We are grateful to all

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Leucistic Adult Female Green Sea Turtles (*Chelonia mydas*) Successfully Nesting at Tortuguero, Costa Rica

Jaime Restrepo¹ & Roldán A. Valverde^{1,2}

¹Sea Turtle Conservancy, Tortuguero, Costa Rica (coordinator@conserveturtles.org)

²Southeastern Louisiana University, Hammond, LA, USA (roldan@conserveturtles.org)

Tortuguero National Park (TNP) is known for hosting the largest nesting population of green sea turtles (*Chelonia mydas*) in the Atlantic Ocean (Carr *et al.* 1978; Tröeng & Rankin 2005). This population's high reproductive success is responsible for the production of millions of hatchlings every year (Fowler 1979). The presence of genetic abnormalities and hereditary defects is not uncommon in hatchlings from this beach, and in sea turtles in general (Godfrey & Mrosovsky 1995; Türkozan & Durmuş 2001; Hitchins & Bourquin 2006; Sönmez & Özdilek 2011; Kaska & Downie 2013). Congenital characteristics such as supernumerary scutes, albinism, or leucism are often present in green turtle embryos and hatchlings. The development and survival of these hatchlings to reproductive age is a topic of which we have little knowledge. Although apparently normal, essential functions such as thermoregulation, navigation, and crypsis may be compromised in individuals lacking pigmentation (Türkozan & Durmuş 2001; Sönmez & Özdilek 2011). The aim of this publication is to report the encounter of two different leucistic female green sea turtles that successfully nested at TNP in 2018, a first-time record for this colony, and to examine the success of these clutches and hatchling development to identify any possible abnormalities.

Every year during the sea turtle nesting season we conduct nightly surveys between the months of June and October to tag, identify, and examine female green sea turtles that come to shore at TNP to lay eggs. As part of our monitoring program, every season we randomly select 200 individuals to mark their nests to evaluate their hatching and emergence success. For every one of these selected females, we perform a detailed egg count and record clutch size, which is used as the basis to estimate success rates of the nests. Nests are marked discretely by triangulation to prevent poaching. Also, nests are monitored daily throughout the incubation period. Once there is any evidence of hatchling emergence, the nests are exhumed, and hatching and emergence success are determined based on the number of collected empty shells, and any abnormalities in embryos and hatchlings are recorded.

Following the above procedures, during the 2018 nesting season two separate adult female green sea turtles with abnormal coloration were detected while nesting. Although they were not albino, the encountered females presented unusually pale complexions. These turtles were examined in detail, tagged and measured. Curved carapace length (± 0.1 cm) was measured three times for each

individual with a measuring tape by the same field assistant, until all three measurements were no more than one cm apart. No other abnormalities besides the lack of pigmentation in their skin and carapace were detected. As per our standard operating procedures, each nest site was marked and monitored closely during the incubation period. In addition, the clutches were protected with plastic nets to prevent predation by dogs, raccoons or armadillos. After 60 days of incubation, the nests were exhumed to determine hatching success. The offspring were examined to identify any possible malformations or abnormal hereditary characteristics.

The first leucistic female green turtle was encountered laying eggs on the north end of Tortuguero's beach, 2.6 km south from the river mouth on 2 October. Four days later on 6 October, the second female nested 3 km south of the first location (see cover photos). Both females completed the nesting process without disturbance. Mean curved carapace length was 108.4 cm for the first and 109.7 cm for the second turtle (± 0.2 cm each). Both were within the normal size range of green turtles at Tortuguero. Neither turtle presented an abnormal number of scutes. Each clutch contained over 110 eggs, the average number for the TNP green turtle colony, and had a hatching success of 94.7% and 90.3%, respectively (Table 1). Those values are seemingly higher than the 82.8% mean hatching success reported for this beach in the past (Fowler 1979).

During exhumation, we found 125 empty shells, 7 unhatched eggs, and 34 hatchlings left in the chamber of the first nest. For the second nest, we found 112 empty shells, 12 unhatched eggs, and 90 hatchlings remaining in the chamber. We measured the straight carapace length (SCL; ± 0.05 cm) with a caliper of each individual hatchling that we observed and did a close evaluation of their body condition, looking for abnormalities or congenital alterations. We obtained an average maximum SCL of 5.20 ± 0.20 cm. We compared this with a sample of hatchlings taken from three random nests marked during the season, from females without visible morphological abnormalities. There was no significant difference in SCL between the two groups (Student's t-test, $T = -1.6$, $df = 38$, $p = 0.119$), and SCL in the leucistic hatchlings ranged from 4.80-5.40 cm. These measurements were seemingly larger than those reported for green turtles in Cyprus hatcheries (Özdemir & Türkozan 2006). Though most of the hatchlings appeared to be normal in size, shape and coloration, we recorded two with malformations of the carapace, where a few marginal scutes were missing (Fig. 1a). We

	Clutch Size	Hatching success (%)	Hatchlings Examined	Deformed Carapace (%)	Scute Variations (%)	Pigmentation Abnormalities (%)
Female 1	132	94.7	34	5.9	8.8	8.8
Female 2	124	90.3	90	0.0	1.1	22.2
Total	256	92.6	124	1.6	3.2	18.6

Table 1. Exhumation results and hatchling malformation classification for nests laid by leucistic green turtles at Tortuguero, Costa Rica.

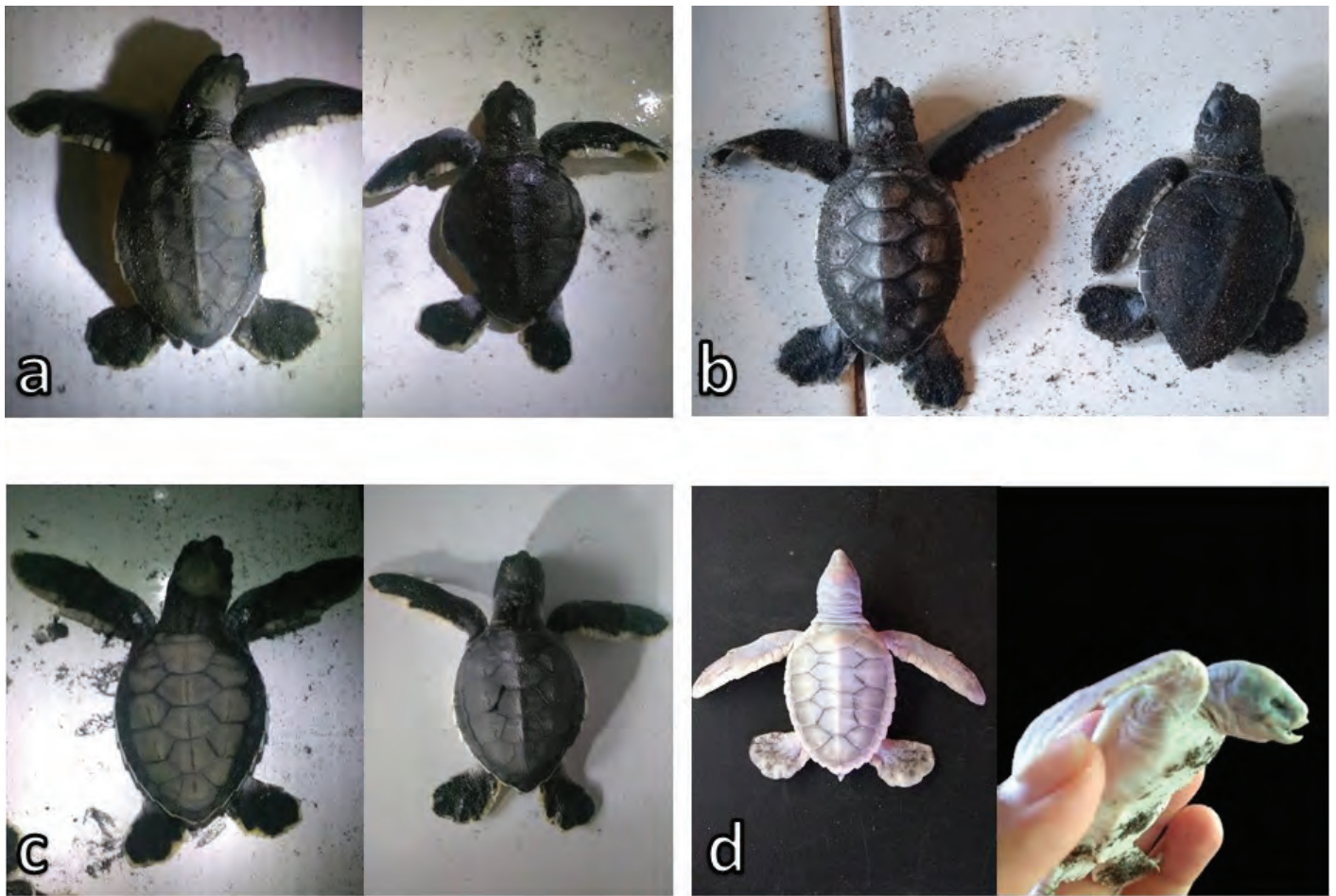


Figure 1. Abnormal hatchlings from clutches of leucistic mothers. a) Hatchlings presenting carapace deformities. b) Contrast between a hatchling with evident lack of pigmentation on its carapace and a hatchling with standard characteristics. c) Hatchlings presenting supernumerary scutes on the rear section of their carapace. d) Leucistic, eyeless, deformed hatchling (dorsal and lateral). Photos: Jaime Restrepo.

observed a remarkable pale coloration in 18.6% of the hatchlings evaluated (Table 1). The lack of coloration was most evident on the ridge, across the vertebral scutes and on the back of the head (Fig. 1b). It is somewhat common among marine turtles to find one or two hatchlings with pigmentation anomalies from a single clutch; however, the lack of pigmentation in several hatchlings is rare (Perrault & Coppentrath 2019). Supernumerary scutes were detected in four individuals, mainly affecting the posterior carapace (Fig. 1c). This condition is not rare in green turtles, even under controlled laboratory conditions (Özdemir & Türkozan 2006). Scute variation on hatchlings from a single nest estimated for these cases (Table 1), was significantly lower than those presented in similar studies (Özdemir & Türkozan 2006; Zimm *et al.* 2017). Though scute abnormalities are more common in albino individuals, their presence does not necessarily affect the development or fitness of the hatchlings (Sönmez & Özdilek 2011). In the second clutch, we encountered a live, active leucistic hatchling ready to emerge from the nest. Besides being leucistic, this individual presented several deformities of the carapace and the head. It had an absolute absence of eyeballs and snout (Fig. 1d). Previous reports of albino hatchlings have shown little to no successful emergence in natural conditions (Türkozan & Durmuş 2001; Perrault & Coppentrath 2019),

though recently up to one fourth of a green turtle clutch resulted in presumably healthy albino hatchlings (Perrault & Coppentrath 2019). Although this eyeless individual may have had the energy and stamina to crawl to the sea, its sight limitation, head deformities, and coloration made it seemingly vulnerable to predation. In fact, this individual wandered on the beach in circles. For these reasons, we sacrificed the individual by placing it in a freezer at -20°C , as recommended (Lillywhite *et al.* 2016).

The two encounters with leucistic adult females constitute the first reports of adult, reproductively active green sea turtles at TNP with evidence of leucism, with a brief description of the fate of their offspring. Interestingly, there is a report of an adult “amelanic” loggerhead sea turtle nesting in the South Queensland, Australia (Limpus *et al.* 1979). Incubation of a few eggs from this female resulted in the production of normally pigmented hatchlings. However, inspection of eggs left in random nests on the beach revealed the occasional presence of dead leucistic embryos that were incapable of tearing the eggshell, presumably due to the lack of the caruncle, a deformity that may have been associated with the lack of coloration (Limpus *et al.* 1979). These reports not only show that leucistic individuals can survive to adulthood, but that they can become reproductively active and contribute to the population.

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First report of *Lepidochelys olivacea* feeding on *Hippocampus patagonicus* in Brazil

Adriana Mastrangelli¹, Rosana Silveira², Mariana Burato¹, Paula Baldassin^{1,3} & Max Rondon Werneck³

¹CTA Serviços em Meio Ambiente, Pontal de Camburi, Vitória, ES, 29062-030, Brazil (E-mail: dri.mastrangelli@hotmail.com; maryburato@hotmail.com; pauletsbj@gmail.com); ²Projeto Hippocampus, Porto de Galinhas, Ipojuca, PE, 55590-000, Brazil (E-mail: info@projetohippocampus.org); ³BW Veterinary Consulting, Praia Seca, Araruama, RJ 28970-000, Brazil (E-mail: max@bwvet.com.br)

The olive ridley turtle (*Lepidochelys olivacea*) is one of the smallest species of sea turtle. It is distributed in tropical and subtropical regions of the Atlantic, Pacific and Indian Oceans and is considered Vulnerable on the global scale (www.redlist.org). The olive ridley turtle is found along the entire coast of Brazil (Reis *et al.* 2010), but primarily nests on beaches along the coast of the northeastern states of Bahia and Sergipe (Silva *et al.* 2007).

Seahorses (Syngnathidae: *Hippocampus*) are a group of boney fishes composed of a large number of species, including many synonymies (Lourie *et al.* 2004). The occurrence of only two species of seahorses was reported in Brazil until a few years ago: *Hippocampus reidi* (found in estuaries and the ocean) and *Hippocampus erectus* (found in the ocean). However, a third morphotype was found in the environment and collections, causing taxonomic confusion. Recently, the relationships were resolved based on morphometric and genetic analyses, leading to the conclusion that three species of seahorse are found in Brazil: *H. reidi*, *H. erectus* and *Hippocampus patagonicus* (Silveira *et al.* 2014).

Although seahorses may make up the diet of sea turtles (Kleiber *et al.* 2010), no events of this interaction have previously been reported for *L. olivacea*. Therefore, this note offers the first documentation

of the consumption of seahorses by an olive ridley sea turtle on the coast of Brazil.

On 14 June 2017, a female *L. olivacea*, measuring 81.6 cm curved carapace length and weighing 25.6 kg, was found stranded dead on Itaipuaçu Beach (-22.97044 °S, -42.95739 °W) in the municipality of Saquarema in the state of Rio de Janeiro, Brazil. The turtle was in good body condition, and during necropsy, four specimens of seahorse were found in the small intestine (Fig. 1 and Fig. 2). Two specimens were partially digested, with the loss of anatomic structures necessary for their identification, such as the snout. The other two specimens examined had sufficient information about snout length (Silveira 2000; Figure 2 A and B), which can be used to document species.

We used the criteria proposed by Silveira *et al.* (2014) to identify species. The head to snout ratio (He/Sn in cm) was considered the main characteristic, as the three species of seahorse in Brazil differ greatly in snout size. *H. reidi* has a long snout (He/Sn: 2.0 to 2.5), *H. erectus* has an intermediate snout size (He/Sn: 2.5 to 3.2) and *H. patagonicus* has the shortest snout of the three (He/Sn: 2.85 to 3.9). Although the specimens examined had lost the maxilla and mandibles, the presence of rostral cartilage attached to the

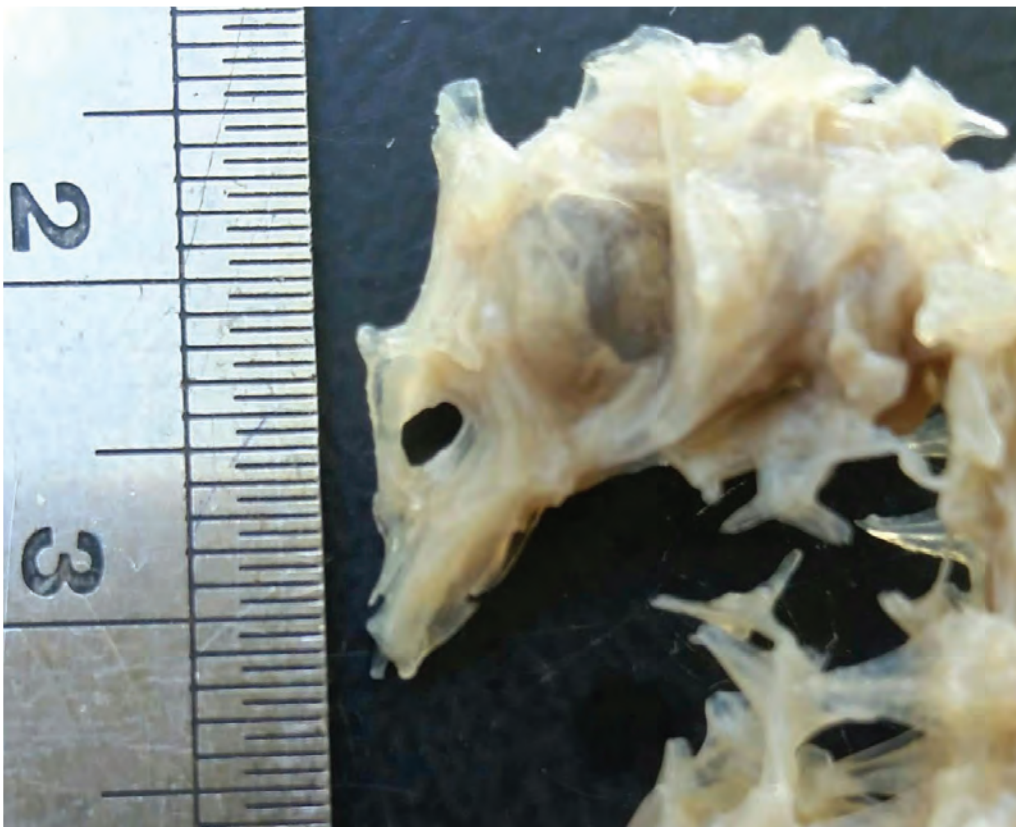


Figure 1. *Hippocampus patagonicus* specimen found in the stomach of *Lepidochelys olivacea* in the state of Rio de Janeiro.

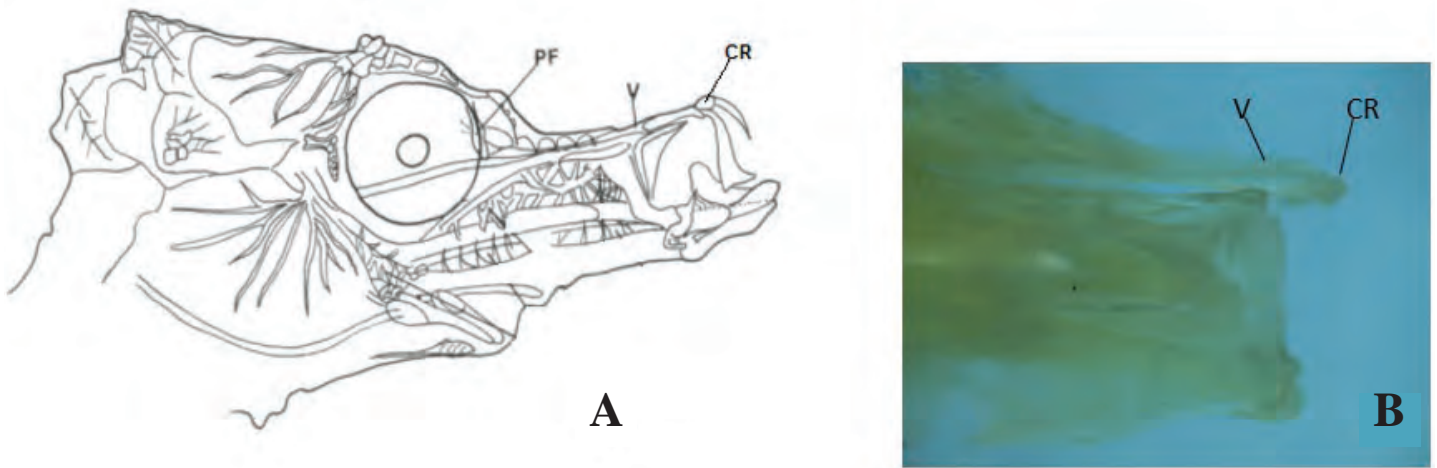


Figure 2. A. Osteological schematic of seahorse showing bones of snout: V, vomer; CR, rostral cartilage (adapted from Silveira, 2000). B. Photomicrograph of snout of seahorse identified as *Hippocampus patagonicus* (magnification: 25 X).

mesethmoid and vomer bone indicated the end of the snout (Silveira 2000; Fig. 2A and Fig 2B), enabling the determination of the $He/Sn = 3.5$, confirming the species as *H. patagonicus*.

Kleiber *et al.* (2010) reported on the occurrence of *H. erectus* and *H. hippocampus* in the digestive track of loggerhead turtles (*Caretta caretta*) from the Atlantic Ocean and Mediterranean Sea and *H. erectus* in Kemp's ridley turtle (*Lepidochelys kempii*) from the West Atlantic Ocean. Given the similarity of diets for both *Lepidochelys* sea turtle species (Marquez 1990), it is not surprising that *L. olivacea* also eats *Hippocampus* spp, although we were unable to find any records in the published literature.

For Brazil, Colman *et al.* (2014) analyzed the diet of 30 individuals of *L. olivacea* on the coast of the state of Sergipe (northeastern region of the country) and described 13 different food items representatives of the phyla Arthropoda (five families of the class Malacostraca), Chordata (three families of the class Osteichthyes) and Mollusca (one family of the class Bivalva and one family of the class Cephalopoda). However, the authors did not describe the occurrence of seahorses in the stomach contents of the turtles analyzed. Therefore, the present note is the first documentation of ingestion of seahorses in *L. olivacea* in the world, broadening knowledge on this predator and its diet in Brazilian waters.

Acknowledgments. Biological samples were obtained through the "Phase 2" beach monitoring project in the state of Rio de Janeiro, Brazil, which is a requirement established by the federal environmental licensing division of the Brazilian environmental agency (IBAMA), for the exploration of oil and gas by Petrobras at the Santos Basin pre-salt province. BW is a company involved with the veterinary medicine activities develops specific consulting work. One of the aims of the company is to disseminate the results of scientific studies to contribute to the conservation of marine organisms.

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REPORT

President's Report For 39th Annual Symposium On Sea Turtle Biology And Conservation, Charleston, South Carolina, USA, 2-8 February 2019

Kenneth J. Lohmann

*International Sea Turtle Society (ISTS); University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
(E-mail: KLOhmann@email.unc.edu)*

The 39th International Sea Turtle Symposium was held in Charleston, South Carolina, USA from 2-8 February 2019. The theme of the Symposium was "Navigating the Future." This forward-looking theme encouraged us to envision future conservation problems before they arise, acquaint ourselves with the emerging frontiers of sea turtle biology, and honor our covenant to the natural world by steering our way toward a bright future for sea turtles and humanity. The symposium featured a record number of workshops (25) encompassing a wide range of topics, as well as a full suite of regional meetings, which allowed participants to discuss region-specific issues. The main days of the symposium featured over 400 oral and poster presentations in eight traditional categories, as well as four special sessions unique to the 2019 symposium. The meeting provided abundant opportunities to socialize with old friends and to meet new ones. In addition to the opening social and the traditional farewell banquet, a host of evening events were held, including film night, the student mixer, speed-chatting with the experts, the dance-your-research competition, the sea turtle trading post, and the live and silent auctions. The Symposium attracted approximately 830 registrants from 52 different countries around the world.

Logo. The logo for the 2019 International Sea Turtle Symposium combined a hatchling turtle with a compass rose and symbolized the theme of the symposium: Navigating the Future (Fig. 1). The logo had additional meaning for the University of North Carolina research team headed by Professor Ken Lohmann (2019 ISTS President) and Professor Cathy Lohmann. For them, the emblem also celebrated

thirty years of research on the mystery of how sea turtles navigate, with the compass representing the magnetic sense of turtles and the central role it plays in long-distance navigation and natal homing. The logo was designed and drawn by Dawn Witherington.

Going Green - Zero Waste. Efforts were made to make the symposium more environmentally friendly. No plastic was used at symposium coffee breaks or other events. Instead of paper mailings, announcements were distributed through e-mail lists, the ISTS website and various social networks. All registrants were also provided with a free souvenir coffee mug emblazoned with the symposium logo for use during coffee breaks. The hotel that served as the symposium venue also has environmentally friendly policies in place, including the following:

JHM Hotels, owner of the Charleston Marriott, partners with *Clean the World* to recycle soap and other toiletries to help the environment and to help those in need.

All lighting was recently replaced with LEDs throughout the hotel.

Recycling, co-mingle, and composting programs were in place. Motion-sensing thermostats are currently being installed in all guest rooms.

Towel and linen recycling programs exist.

Paper straws have replaced plastic straws throughout the hotel.

Recycle receptacles are located in each guestroom.

The College of Charleston's Zero Waste Program also volunteered for the symposium with the goal of making the 2019 ISTS a zero-waste conference. The Zero Waste Program is managed by the College of Charleston's Office of Sustainability, which works closely with the College's Dining Services (Aramark) and the organics hauling company SMART Recycling to divert as much organic waste from the landfill as possible. The Zero Waste Team consists of College of Charleston undergraduates and recent graduates. We thank Dr. Ashley Lavender for arranging this partnership between the ISTS and the College of Charleston.

Workshops and Regional Meetings. Workshops and regional meetings were scheduled during the three days prior to the main four-day symposium. These early events provided participants with opportunities to share research techniques and exchange information about environmental and sea turtle conservation issues. The workshops and meetings attracted a large number of attendees and were highly successful. A total of twenty-five workshops were held: (1) Sea Turtle Rehabilitation and Veterinary Medicine; (2) Unmanned Aerial Vehicles (Drones): Problem-solving, Turnkey Systems, and What's Next; (3) Captive Rearing for Research and Conservation; (4) Does What We Do Matter? The Critical Need to Evaluate and How To Do It; (5) Geographic Information System; (6)



Figure 1. The logo for the 2019 International Sea Turtle Symposium.



Figure 2. Participants of the Mediterranean Regional meeting. Photo by Anna Stamatidou.

Advanced Veterinary Techniques; (7) Migratory Connectivity in the Ocean: Global Sea Turtle Migratory Routes and Connected Areas; (8) Temperature-dependent Sex Determination: From Molecular Biology to Evolutionary Ecology; (9) Reproductive Physiology: What Turtle Gonads Reveal and How to Read Their Secrets; (10) Recreational Fishing Interactions with Sea Turtles; (11) Beyond the Brochure: Creating a Meaningful Education Component to Help Secure your Research Grant; (12) Global Perspectives on Photo ID as a 21st Century Tool for Sea Turtle Research and Conservation; (13) Getting the Most out of Satellite Telemetry for Marine Turtles; (14) Marine Debris and Sea Turtles; (15) Environmental Contaminants and Sea Turtles; (16) Tortoiseshell Trade: Current Status and Outreach Effort; (17) Finding the Solution for Light Pollution through Collaborative Management; (18) How Engineered Beaches Change Sea Turtle Nesting and Incubation; (19) Measuring and Mitigating Rising Nest Temperatures; (20) Assessing Trends in the Biodiversity of Marine Turtles from 2010-2020 to Contribute to the 2nd United Nations World Ocean Assessments; (21) Eastern Pacific Leatherback Workshop; (22) The Global Male Sea Turtle Initiative: Adding Males to the Conservation Equation; (23) How to Fund Sea Turtle Conservation Projects; (24) Updates on the Status of Sea Turtles Worldwide and How Proposed Changes to the U.S. Endangered Species Act Might Affect Them; and (25) Trending Research Topics and Techniques (led by the Student Committee.) In addition to the workshops, seven regional meetings were also held, allowing participants from around the world to discuss specific problems and challenges impacting their regions. These regional meetings included Africa, East Asia, Indian Ocean and South East Asia (IOSEA), Mediterranean (Fig. 2), Pacific Islands/Oceania; Latin America (RETOMALA), and the IUCN Marine Turtle Specialist Group (MTSG).

Main Symposium Program. The main symposium began with opening remarks from the 2019 ISTS President Ken Lohmann, followed by three keynote speakers. Elena Mustakova-Possardt addressed attendees on the topic of “Meeting the Future with Constructive Resilience.” Elena is an educator, social scientist, and former professor in adult developmental psychology, as well as a counselor and psychotherapist in private practice in the metro DC area. Her talk focused on the need to face political, social, and environmental challenges with constructive resilience that allows us to persevere through upheaval and setbacks. Colin Limpus, who serves as Chief Scientist of the Aquatic Species Program in

Queensland, Australia, presented the second keynote address, titled “Fifty Years of Walking with Turtles: Looking Forward from Down Under.” This talk highlighted important events in the history of sea turtle biology and conservation, as well as lessons for the future. Sally Murphy, former head of the South Carolina sea turtle program, concluded the opening session by providing a brief reading from her newly published memoir *Turning the Tide*. She also shared amusing anecdotes from the early days of the ISTS.

The Symposium program of oral and poster presentations ran from Tuesday, February 5th through Friday, February 8th. Program Chairs John Wang, Kate Mansfield, and Nathan Putman, along with Poster Chair Larisa Avens and 38 Session Chairs, developed an amazing symposium program consisting of 172 oral papers and 232 posters. Many of the presentations were within the traditional eight session categories: (1) Anatomy, Physiology and Health; (2) In-Water Biology; (3) Nesting Biology; (4) Population Biology and Monitoring; (5) Fisheries and Threats; (6) Conservation, Management and Policy; (7) Education, Outreach and Advocacy; and (8) Social, Economic and Cultural Studies. In addition to the traditional regular sessions, four special sessions were also held: “Genetics and Genomics of Sea Turtles, from Research to Conservation”; “Using Science to Inform Conservation Policy: Past, Present and Future”; “Navigation, Migration, and Natal Homing”; and “The Future of Sea Turtle Conservation.”

Film Night. Chairs Tom Backof, Christian Gredzens and Katherine Comer Santos gathered 22 short films highlighting elements of sea turtle research, conservation and outreach from diverse geographic areas, including Florida, Surinam, Italy, California, Papua, Ghana, Argentina, Gulf of Mexico, Texas, Brazil, and Costa Rica. The films were shown publicly during one evening of the symposium.

Student Committee. The Student Committee (chaired by Katherine Shaw, Christopher Gatto and Itzel Sifuentes) coordinated activities designed to welcome and assist student attendees. Three activities were undertaken. The first was “Student Presentation Feedback”, in which 81 volunteer evaluators provided feedback and suggestions for 157 student presentations. The second activity was a Workshop titled “Trending Research Topics and Techniques.” This workshop covered a range of different techniques that are currently at the forefront of sea turtle research. Experts on these techniques (Richard Reina, Ryan Chabot, Brian Shamblin, Boris Tezak, Franciscus Scheelings and Camryn Allen) shared information with 25 attendees on how these techniques were developed, how they are currently being used, the methodology behind the techniques, and how the techniques might potentially be used in the future. Lastly, with the goal of promoting networking and communication among students and other symposium participants, the Student Social Mixer event was held together with “Speed Chatting with the Experts” and the new “Sea Turtle Trading Post.” A brief description of these events follows.

Speed-Chatting with the Experts. For a small fee, symposium attendees had the opportunity to have one-on-one chats with top research and conservation experts. All funds raised went to support the symposium travel grant fund. Nine experts shared their knowledge and experiences in 5-minute slots in themes such as community-based conservation, bycatch reduction, genomics and ecophysiology, endocrinology and sex identification, spatial and movement ecology, human dimensions of sea turtle conservation,



Figure 3. Participants of Speed-Chatting with the Experts. Photo by Austin Fitzhenry.

animal behavior and magnetic navigation, interactions between animals and environment, population biology and TSD, and conservation using genetic tools. The experts were: Jesse Senko, Lisa Komoroske, Camryn Allen, Dan Evans, Natalie Wildermann, Catherine Lohmann, Nathan Putman, Marc Girondot, and Carlos Carreras Huergo (Fig. 3).

Sea Turtle Trading Post. Many groups that work on sea turtle biology and conservation have surplus equipment and supplies that are no longer needed. A new event was therefore developed to find new homes for this equipment. The sea turtle community was invited to donate new or gently used field and lab equipment, and people interested in acquiring each item of equipment could enter their names in a lottery. The beneficiaries were primarily students, as well as projects from low-income countries. Donated items included GPS gear, field notebooks, tape measures, markers, headlamps, cameras, rain ponchos, and insect repellent. This first year was a resounding success and the event will be continued and expanded in future symposia.

Social Events. The social component of the symposium included the Welcome Social, Student Committee activities, the Silent and Live Auctions, and the Award Ceremony and Banquet. The Welcome Social was held at the South Carolina Aquarium. Participants were able to enjoy drinks and Charleston cuisine while viewing the numerous marine animals and exhibits. The Silent and Live Auctions, as usual, were among the most popular events. Auctioneers Larry Wood and Marina Zucchini were pleased to announce that the events jointly raised approximately US\$22,000 to help ISTS members attend future symposia via travel grants.

The banquet was held in the evening of the final day of the symposium. Events included the Award Ceremony in which numerous awards were presented, including the Archie Carr Student Award, the ISTS Career Awards and the Grassroots Conservation Award. The formal portion of the evening closed with words of appreciation from the President and the ceremonial passing of the ISTS Presidential Trowel to incoming 2020 President Diego Amoroch. Afterwards there was music and dancing to celebrate the end of the symposium.

ISTS Career Awards. Erin Seney, ISTS Career Awards Committee chair, and her committee (Shaya Honarvar, Sheryan Epperly and Irene Kelly) presented awards to an incredible group of recipients. *2019 ISTS Lifetime Achievement Awards* were presented to Michael Salmon (USA), Eng Heng Chan (Malaysia), René

Márquez-Millán (Mexico), and Jeffrey Miller (USA). Recipients of the *2019 ISTS Champions Awards* were the Family Island Research and Education Foundation (Bahamas), Jeannie Martin (USA), Wallace J. Nichols (USA), and Jeanette Wyneken (USA). Roderic Mast (USA) was the recipient of the *Ed Drane Award for Volunteerism*. Additionally, ISTS President Ken Lohmann presented the *2019 President's Awards* to the Bald Head Island Conservancy (USA) and to the University of Georgia Sea Turtle DNA Fingerprinting Project (USA).

Archie Carr Student Awards. Chairs Matthew Godfrey and Andrea Phillott received requests to consider 121 student presentations (53 orals and 68 posters) for student awards. Numerous judges assisted with evaluation of presentations, including Aliko Panagopoulou, Amanda Southwood-Williard, Ana Barragan, Bibi Santridian Tomillo, Craig Harms, Daphne Wrobel Goldberg, Jennifer Lynch, Marc Girondot, Mark Dodd, Mark Roberts, Mike James, Qamar Schuyler, Rupika Rajakaruna, Shaya Honarvar, Yakup Kaska and Zoe Meletis. Eight students were recognized for outstanding presentations. In *Biology*: Boris Tezak (oral winner), Kayla Goforth (oral runner-up), Robert Johnson (poster winner), MacKenzie Tackett (poster runner-up). In *Conservation*: Alessandra Bielli (oral winner), Emily Duncan (oral runner-up), Katie Mascovich (poster winner) and Mia El-Khazen (poster runner-up).

Grassroots Conservation Award. The judges (Alejandro Fallabrino, Angela Formia, Jack Frazier, Milagros Lopez, Muralidharan Manoharakrishnan, Wallace J. Nichols, Manjula Tiwari and Ingrid Yanez) evaluated the self-nominated presentations. The award was given to *Sea Turtle Conservation Curacao* for their presentation “*Effective Conservation through Partnership with Precious Plastic Initiative.*”

Dance-Your-Research Competition. The Dance-Your-Research Competition was held for the second time. This year, the Golden Hawaiianas (Flip-Flops) were awarded to Leyna Stemle, Phillip Allman and Andrew Agyekumhene from Ghana Turtle Conservation Project for the dance performance representing their research “*Nesting Sea Turtles in Ghana, West Africa*” (Fig. 4). Their research involved estimating the number of nests produced by each sea turtle species in their work area, evaluating the spatial distribution of nests, and gaining insights into hatching success. In the submitted video,



Figure 4. Phillip Allman and Andrew Agyekumhene posing with the Golden Hawaiianas award. Photo by Kate Mansfield.

children from University Practice South Special School danced to drums while portraying sea turtles going through all stages of the nesting process, including swimming to shore, crawling onto the beach, digging the nest, laying eggs, covering the nest, and reentering the ocean.

Exhibitors and Vendors. Chair Janet Hochella coordinated 25 exhibitor and vendor displays that attendees visited throughout the week. The exhibitors included: Barbara's Sweetgrass Baskets; Bioko Marine Turtle Program; Casa de Coco Conservation Project; C&W Energy Solutions; Cefas Technology Limited; Center for Biological Diversity; Holbrook Travel; Karumbé; The Leatherback Trust; Lotek; Malama na Honu; Marine Life Alliance; National Marine Fisheries Service; Oceanic Society; Sea Turtle Conservancy; The Ocean Foundation's St. Croix Leatherback Project and Boyd Lyon Sea Turtle Research Fund; St. Kitts Sea Turtle Monitoring Network; SEE Turtles; Telonics Inc.; Turning the Tide (memoir by Sally Murphy); Turtle Conservation Project Sri Lanka; Turtles In Clay; U.S. Navy Stewards of the Sea; Widecast; and Wildlife Computers.

Closing Session. The symposium closed with a special session featuring two keynote addresses. The first was by Blair Witherington and titled "What's the Point of Sea Turtle Conservation When We're All Going to Die?." The second was by Wallace J. Nichols, titled "Sea Turtles are Medicine." Final closing remarks were made by Ken Lohmann, ISTS President.

Society Business Plenary. The 2019 ISTS Business Meeting was held on Friday, February 8th (Fig. 5). ISTS President Ken Lohmann called the meeting to order and reports were provided by the Treasurer, Travel Grants Committee, Nominations Committee, and Student Committee. Other issues related to the Society were also discussed. No resolutions were submitted for consideration at this Symposium. Diego Amoroch, 2020 ISTS President, provided information regarding next year's symposium, which will be held in Cartagena, Colombia. The theme of next year's meeting is "Vision 20/20" and the symposium will be held March 14-20, 2020.

ISTS Elections. Kellie Pendoley from Australia was elected President for the 2021 symposium. The elections also added two new members to the Board of Directors (year indicates board member's end of term): Maria Angela "Neca" Marcovaldi (2024) and Marco Garcia-Cruz (2024). Roldan Valverde, Mustapha Aksissou and Gabriela Velez-Rubio were elected to join the Nominating Committee. Hector Barrios-Garrido joined the Career Awards Committee.



Figure 5. Symposium attendees during the closing session. Photo by College of Charleston.

Travel Grants. Making the symposium accessible to students and international participants is a priority of the Society, and to this end travel grants are provided to offset the cost of attending. Alexander Gaos chaired the Travel Grant Committee. Regional Travel Chairs were Karen Eckert, Alejandro Fallabrino, Angela Formia, Emma Harrison, Aliko Panagopoulou, Andrea Phillott, ALan Rees, and Kelly Stewart. The ISTS, with assistance from The Conservation Foundation, was able to support a total of 127 travel grant applicants with full lodging during the symposium. The distribution of bed grants per region was as follows: six to Africa representatives; six to Southeast Asia/Pacific; seven to South Asia; 11 to Europe; three to Caribbean; 24 to Mexico and Central America; 27 to South America; and 43 to US and Canada.

Funding. Generous funding by many organizations and individuals contributed to the success of the 2019 ISTS. The organizing committee deeply thanks the following donors for their generosity. *Gold level (\$10,000-\$24,999):* Ripley's Aquariums (Ripley Entertainment, Inc.). *Silver level (\$5,000-\$9,999):* The Conservation Foundation; Sea Turtle Conservancy; Wildlife Computers; Lohmann Lab (UNC-Chapel Hill); University of North Carolina College of Arts and Sciences; and Ocean Conservancy. *Bronze level (\$1,000-\$4,999):* National Oceanic and Atmospheric Administration (NOAA); South Carolina Aquarium; Lotek; Gumbo Limbo Nature Center; National Save The Sea Turtle Foundation; George H. Balazs & Golden Honu Services of Oceania; Turtle Hospital; Virginia Aquarium & Marine Science Center; Sea Turtle Association of Japan; Beyond Protection; Network for Endangered Sea Turtles (NEST); New England Aquarium; Coastal Wildlife Club, Inc.; Texas State Aquarium; Karen Beasley Sea Turtle Rescue and Rehabilitation Center; Loggerhead MarineLife Center; and Kona Brewery Company. *Aluminum level (\$500-\$999):* The Leatherback Trust; Turtle Time, Inc; Southeast Regional Sea Turtle Network (SERSTN); Ecological Associates, Inc.; Georgia Sea Turtle Center; LGL Ecological Research Associates; College of Charleston Office of Sustainability; and Mike & Jeanette. *Inconel level (\$25-\$499):* Gabriella Carvajal; David Fittinghoff; Nicholas & Sandra Fittinghoff; Kymberlee Haynes Castillo; Janet Hochella; Kate Mansfield; Sandra Pfeifer; Lisa Rodriguez; Erin Seney; Debbie Sobel; Kelly Stewart; and Jeanette Wyneken. We are also grateful to those who donated items for the auctions and to those who bid on them.

Acknowledgments. Organising and putting on the Charleston symposium required an immense team effort. It could not have been accomplished without a small army of dedicated members willing to take on leadership roles, serve on committees, and assist with diverse tasks such as registration, workshop organization, fundraising, arranging travel grants, and putting on the auctions. By alphabetical order of their first name, the Society thanks Alan Bolten, ALan Rees, Alejandra Rios, Alejandro Fallabrino, Alexander Gaos, Alexis Guilleux, Aliko Panagopoulou, Amanda Southwood-Williard, Ana Barragan, Andrea Phillott, Andres Estrades, Andrew DiMatteo, Andrew Agyekumhene, Angela Formia, Ann Marie Lauritsen, Antonio Di Bello, Ashley Lynn Lavender, Becca Eaton, Bibi Santridian Tomillo, Blair Bentley, Brad Nahill, Brendan Godley, Brian Shamblin, Brian Stacy, Bruno Giffoni, Bryan Wallace, Camryn Allen, Carlos Carreras Huergo, Carrie Upite, Catherine Kilduff, Catherine Lohmann, Cathi Campbell, Christian Gredzens, Christopher Gatto, Claire Jean, Cody Mott, Colin Hunter, Colin

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Volunteers in North Carolina, USA, check on the hatching success of a loggerhead nest. Photo by M. Godfrey.

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