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Olive ridley arribada on Gahirmatha beach, Odisha, India, with the nearby Maipura river delta in the background. See pages 1-2. Photo: M. Muralidharan.

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Olive Ridleys and River Mouths: Speculations About the Evolution of Nest Site Selection

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Around the world, many olive ridley mass nesting beaches occur near river mouths, including those in India (Shanker *et al.* 2003), Mexico (Ocana *et al.* 2012) and Costa Rica (Cornelius & Robinson 1985). The main nesting beach for Kemp's ridley turtles, in Tamaulipas, Mexico, also has many rivers and estuaries backing it (T. Wibbels pers. comm. 2019). In Odisha State on the east coast of India, all the olive ridley mass nesting beaches are very close to river mouths, Gahirmatha near Maipura River mouth (Bustard 1976), Devi named after its proximity to Devi River (Kar 1982) and Rushikulya (Fig. 1) also named for Rushikulya River (Pandav *et al.* 1994). In Mexico, the mass nesting beach at La Escobilla, perhaps the largest ridley rookery in the world, is adjacent to a river mouth, but other rookeries are not (A. Abreu-Grobois pers. comm. 2020). In Costa Rica, there are river mouths near all the mass nesting beaches, including Nancite, Corozalito and Ostional (R. Arauz and R. Valverde pers. comm. 2020). In Suriname, the mass nesting beach at Eilanti, which hosted arribadas in the 1960s was located next to Marowijne river (Hoekert *et al.* 1996).

Mass nesting at these beaches often occurs within a few kilometers of the river mouth, with only sporadic nesting along the rest of the coastline. Even along large stretches of coast where only sporadic nesting occurs, nesting densities are often higher near river mouths. Tripathy *et al.* (2003) found that the nesting density of olive ridleys on the east coast of India was several times higher within 5 – 10 km of major river mouths than the rest of the coast. On Great Nicobar Island (India) in the Bay of Bengal, olive ridleys share nesting beaches with leatherback turtles, at the mouths of the Rivers Galathea, Alexandria and Dagmar (Andrews *et al.* 2006). This pattern of higher densities of nests near river mouths has also been observed in South America and Africa, including Sergipe in Brazil (J.C. de Castilhos pers. comm. 2019) and in Gabon and the Republic of Congo (K. Metcalfe & B. Godley pers. comm. 2019). At La Flor in Nicaragua, the highest concentrations of nesting occur in front of small estuaries that do not break open during the nesting season (S. Honarvar, pers. comm. 2020).

There is evidence of dynamic shifts in topography at many of these nesting beaches, including at all the mass nesting beaches in Odisha, India. In Gahirmatha to the North, nesting occurred on a several kilometer-long spit, which was part of the mainland coast at the mouth of the River Maipura from the early 1970s onwards when it was first documented (Bustard 1976) till the late 1980s (Pandav *et al.* 1998). In 1989, this spit broke away during a cyclone and formed a separate island where nesting subsequently occurred. This island was then further divided into smaller sand bars during cyclones that occurred in the late 1990s. These sand bars undergo a significant amount of erosion and accretion, which has also resulted in the spatial movement of these islands. In Rushikulya, a monitoring program over the last decade has shown substantial shifts in the

nesting beach, caused by erosion and accretion at the river mouth (Chandarana *et al.* 2017).

A similar pattern of beach erosion and accretion has been observed at the mass nesting beaches in Ostional (Valverde *et al.* 2012) and other locations in Costa Rica (R. Arauz pers. comm. 2019). In Suriname, there have been significant changes in the topography of Eilanti beach caused by the Marowijne river, resulting in shifts in nesting (Hoekert *et al.* 1996; Goverse 2003). Biologists working at many other olive ridley nesting sites have observed that beaches near lagoons and estuaries undergo frequent erosion and accretion (J.C. de Castilhos, Brazil; M. Girondot and V. Plot, French Guiana; K. Metcalfe, Gabon, pers. comm. 2019; K.S., Orissa and Andamans, unpubl. data).

In island systems such as the Andaman and Nicobar Islands, olive ridleys rarely nest on beaches with reefs and rocks in the offshore waters, like green and hawksbill turtles do (Andrews *et al.* 2006). This may be partly due to the fact that, as small animals, they are averse to getting knocked about against hard substrates and injured. Hence, the safest offshore approaches on islands may be on beaches and sand bars created by river mouths. But why do they choose locations near river mouths even on mainland coasts that have vast stretches of sandy beaches?

There are multiple hypotheses for the affinity of olive ridley turtles for beaches near river mouths. I argue that it is most likely related to their mass nesting behavior. Every year, at any mass nesting beach, these turtles lay millions of eggs in a small area, often less than one hundred meters wide, and 2 to 3 km long. A significant proportion of these eggs are destroyed, and rot, leading to an accumulation of soil microbes and fauna. After a few years of nesting at more or less the same site, the potential for infection and infestation could increase dramatically. There is evidence that nest density can affect hatching success at mass nesting beaches (Fonseca *et al.* 2009; Ocana *et al.* 2012) through either increased microbial load (Bezy *et al.* 2015) or its effect on oxygen and carbon dioxide concentrations (Honarvar *et al.* 2008).

What better way to deal with this situation than have nature replace the beach periodically. In Odisha, for example, with the northeast monsoon and seasonal cyclones, the mass nesting beaches experience significant erosion every few years and are sometimes completely destroyed (Chandarana *et al.* 2018). As we have observed, river mouths are dynamic; heavy rains or storms often result in changes in the courses of rivers and in particular, the locations of river mouths. Thus, stretches of beach keep getting washed away on a periodic basis. At the same time, new beaches and sand bars are created that were not present before with the accretion of sand. In effect, the patch of sand with millions of putrefying eggs and/or their microbial communities is washed away and a new beach with clean sand is prepared for the olive ridleys. This

could significantly increase hatching success and productivity and contribute substantially to population recruitment. In one instance, hatching success at Nancite increased significantly after a flooding event (R. Valverde pers. comm. 2020).

There are other hypotheses that may explain this pattern of nest site selection. Sand bars of the type that are found near river mouths may harbor fewer predators. Since olive ridleys lay shallow nests that are susceptible to predation, this could explain the affinity for river mouths in both solitary and mass nesting populations of the species. While there can be on-shore factors influencing nest-site selection, there could be in-water or oceanographic factors as well. Mesoscale oceanographic features such as currents and eddies may also influence the location of mass nesting beaches (Coria-Monter *et al.* 2019) due to their effect on productivity and adult or hatchling energy expenditure. Being near a river mouth may, in particular, aid in hatchling transport away from the beach, which could increase their fitness (see Putman *et al.* 2010, 2012).

Since arribadas may have played a significant role in ridley evolution, through predator satiation, (Eckrich & Owens 1995; Bernardo & Plotkin 2007), it seems compelling that they would select sites where hatching success may be maximized. While I

provide a possible explanation of the evolutionary cause for olive ridley preference for river mouths, there are likely to be proximate cues as well such as salinity, sea surface temperature, moisture, bathymetry or other physiographic features. Future studies should explore both ultimate and proximate causes of nest site selection in olive ridley turtles.

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Figure 1. Tracks from nesting olive ridley sea turtles during an arribada on Rushikulya nesting beach in Odisha, India (photo by Kalyan Varma).

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Loggerhead Turtle Captured in the Rio de la Plata is Found 10 Years Later Nesting in Espírito Santo, Brazil

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An adult female loggerhead turtle (*Caretta caretta* Linnaeus, 1758) bearing a uniquely numbered flipper tag XXZ 684 (Inconel 681 style Tag, National Band and Tag Co.) was found in October 2013 nesting at southeastern Brazil (Fig. 1). The turtle was initially tagged on the right front flipper after being incidentally captured on 03 February 2004 by a Uruguayan trawler operating in the Common Argentinean-Uruguayan Fishing Area (ZCPAU) (Fig.2). At that time, Karumbé was conducting a bycatch monitoring program in that fishery (“Onboard Tagging and Data Collection Program - PROMACODA”, Laporta *et al.* 2012), where trained fishermen volunteered to collect data and samples from incidentally captured sea turtles and tag them before release. The turtle was released 29 nautical miles south of Montevideo, Uruguay (35.3800°W, 55.9500°S), after the fishermen allowed it to fully recover onboard for 5 hours. The PROMACODA project provided evidence that the Uruguayan coastal pair bottom-trawl fishery interacts frequently with sea turtles, and generated data from about 99 loggerhead turtles that were incidentally captured between April 2002 and June 2005. This participatory project involved fishermen and researchers with the aim of increasing the knowledge about the biology of sea turtles while helping to mitigate the bycatch (Laporta, *et al.* 2012).

The same turtle was observed nesting on 15 October 2013 at Comboios beach, state of Espírito Santo, Brazil (19.6768°S, 39.8959°W) during the regular monitoring program of Fundação Projeto TAMAR according to the National Plan of Conservation of Sea Turtles of Centro TAMAR/ICMBio (Brazilian Program for Conservation of Sea Turtles). The minimum distance between the tagging location at the feeding area and the recapture at the nesting beach is 2,400 kilometers. The loggerhead sea turtle is the most common nesting species found along the southern coast of Brazil (Marcovaldi & Laurent 1996). The highest density of loggerhead nests in Espírito Santo state, Brazil, is found around the Doce River mouth during the breeding season, which runs from the end of August to the end of February and peaks between October and December (Barreto *et al.* 2019).

The tag was in bad condition and thus was removed. A new tag was applied on the trailing edge of each front flipper (BR 78089 and BR 78090 - Inconel Tags, National Band and Tag Co.). When recaptured in Brazil the turtle measured 107.0 cm curved carapace length, front nuchal notch to caudal tip (CCLn-t). The measurement taken when the turtle was originally tagged almost ten years before was 82.0 cm CCLn-t, so based on the size distribution of nesting



Figure 1. Tag XXZ 684 found on a loggerhead turtle on 15 October 2013, while nesting on Comboios beach, Brazil.



Figure 2. *Besugo I* crew, releasing tagged loggerheads in Uruguayan waters on 03 February 2004.

females in Comboios (83.0-120.0 cm, Baptistotte *et al.* 2003) this turtle was likely immature when it was captured and first tagged. The curved carapace length growth of this female was 2.5 cm/year, which is faster than that reported by Lenz *et al.* (2016), who estimated a mean annual growth rate of 2.1 cm/year for *C. caretta* in the western South Atlantic Ocean. Both projects use the measurement methods outlined by Bolten (1999).

This is the first record of a juvenile turtle tagged in the Rio de la Plata that was later found nesting in Brazil. However, there are several previous recaptures of adult loggerhead turtles that were found in coastal waters of Uruguay after nesting in Brazil. The first record of this connection, reported by Almeida *et al.* (2000), was a loggerhead turtle tagged while nesting at Pontal de Ipiranga beach, Espírito Santo in November 1991, that was found stranded dead in Punta del Diablo, Uruguay nearly seven and a half years later in April 1999. Afterwards, Laporta & Lopez (2003) reported that a nesting female loggerhead tagged in October 1995 in Arembepe, Bahia, Brazil, was recaptured almost six and a half years later (March 2002) by a Uruguayan bottom trawler operating in the ZCPAU. Coincidentally, this second recapture record sparked the beginnings of what turned out to be the PROMACODA, initially run through Karumbé (2002-2007), and thereafter by CICMAR. Since 2010, five other adult female loggerheads were incidentally captured by Uruguayan coastal bottom trawlers after being tagged while nesting in Brazilian beaches (unpublished data).

Laporta & Lopez (2003) suggest that some Brazilian loggerhead sea turtles may migrate to Uruguayan waters to feed. Although these data are relatively sparse, Almeida *et al.* (2000) suggest that adult sea turtles from Espírito Santo can migrate long distances.

Increased fishing activity in recent years is considered a major threat to SW Atlantic loggerhead turtles, directly affecting the population of juveniles (Sales *et al.* 2008), and subadults and adults feeding on the continental shelf of Rio Grande (Monteiro *et al.* 2016) and Uruguay (Laporta *et al.* 2012). According to Giffoni *et al.* (2014), loggerhead is the most captured sea turtle species by the Brazilian and Uruguayan pelagic longline fleets, and the bycatch rate of both fleets is among those higher worldwide (Pons *et al.* 2010). This strongly suggests that regional and international cooperation can contribute greatly towards the success of sea turtle conservation.

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Dietary Components of Green Turtles in the Lakshadweep Islands, India

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Of the seven extant sea turtles, green turtles (*Chelonia mydas*) are the only species that undergo stark ontogenetic shifts in their diets as they develop (Limpus *et al.* 2005). After their juvenile stage, green turtles move from oceanic to neritic foraging sites and switch their diet from omnivorous to herbivorous (Bjorndal 1997; Hirth 1997; Reich *et al.* 2007). As adults, individuals show a strong preference for seagrasses and algae, with certain populations showing more preference toward one than the other (López-Mendilaharsu *et al.* 2005; Jardim *et al.* 2015; Velez-Rubio *et al.* 2016). However, geographic variability depending on resource availability has been observed in green turtles (André *et al.* 2005; Fuentes *et al.* 2006), for example, low levels of spongivory in the diets of Nicaraguan (Mortimer 1981), Bahamian (Bjorndal 1980; 1990), and Hawaiian populations (Russell *et al.* 2011).

Foraging plays a crucial role in influencing the growth and stability of sea turtles and their populations (Bjorndal 1997). It helps in their somatic growth which consequently affects their rate of maturity and reproductive ability (Arthur & Balazs 2008). A delay in the maturity of individuals could have adverse effects on their survival until adulthood (Balazs & Chaloupka 2004). In addition, the quality and quantity of their foraging resources are also known to impact the inter-annual variability in nesting (Arthur

& Balazs 2008). Therefore, availability and quality of resources determine the diet and consequently, the health of a population.

In the Indian subcontinent, the Lakshadweep islands, located between 8° to 12° N and 71° to 74° E (Fig. 1), serve as foraging and nesting grounds for adult and juvenile green turtles in the northwestern Indian Ocean (Tripathy *et al.* 2002, 2006). The low-lying lagoons of the islands support thriving populations of various seagrasses and algae which, in turn, provide suitable feeding sites for the herbivorous green turtles. It has been observed that of the seven seagrass species found in the Lakshadweep islands, green turtles tend to feed specifically on *Thalassia hemprichii* and *Cymodocea rotundata*, of which *Thalassia* sp. is preferred (Kelkar *et al.* 2013).

Apart from the consumption of these two seagrass species, there is little known about the remainder of green turtle diets in the Lakshadweep islands. Moreover, the green turtle population may have increased in the last 15 years leading to overgrazing of *Thalassia* and *Cymodocea* communities resulting in a shift in species composition in the lagoons (Kelkar *et al.* 2013). This could induce a change in their diets or a change in their foraging grounds. Hence, it is important to determine diet preferences, which will assist in devising a management plan for green turtles

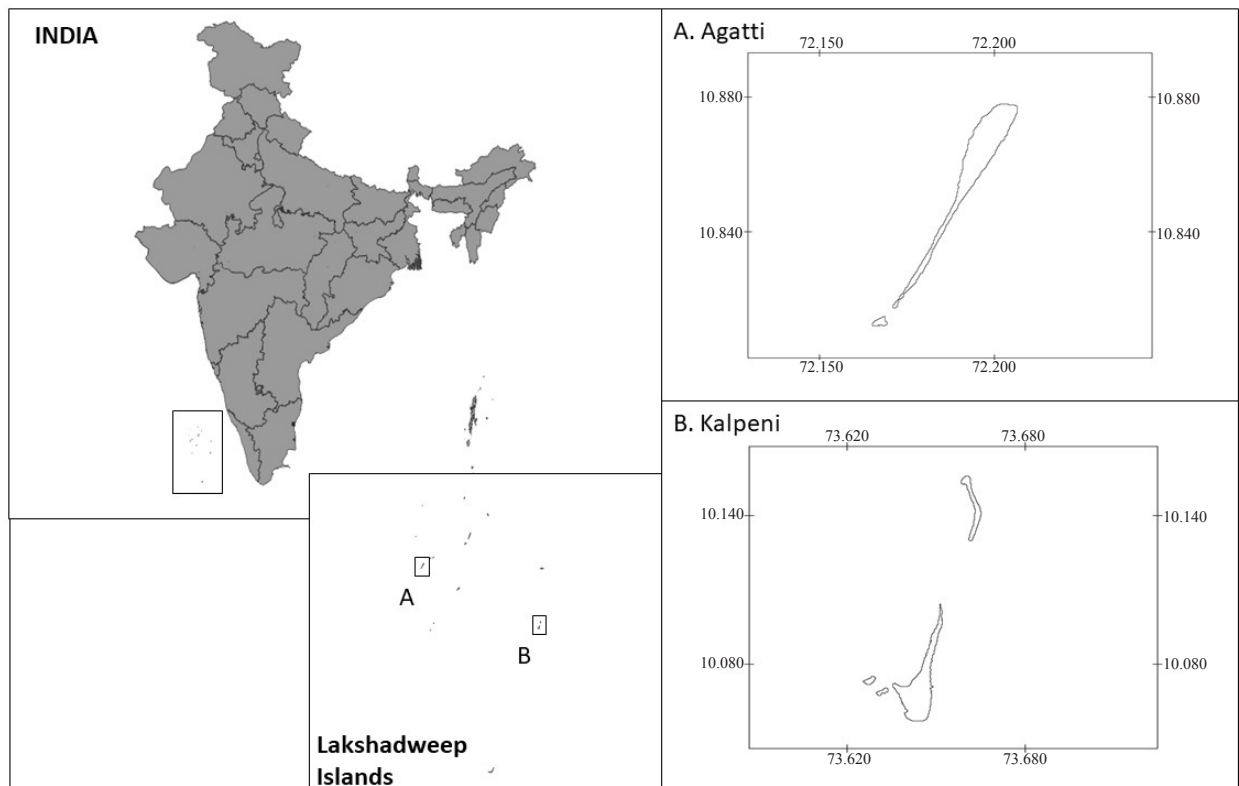


Figure 1. Map of the Lakshadweep islands and the two islands - Agatti and Kalpeni- from where the fecal samples were collected.

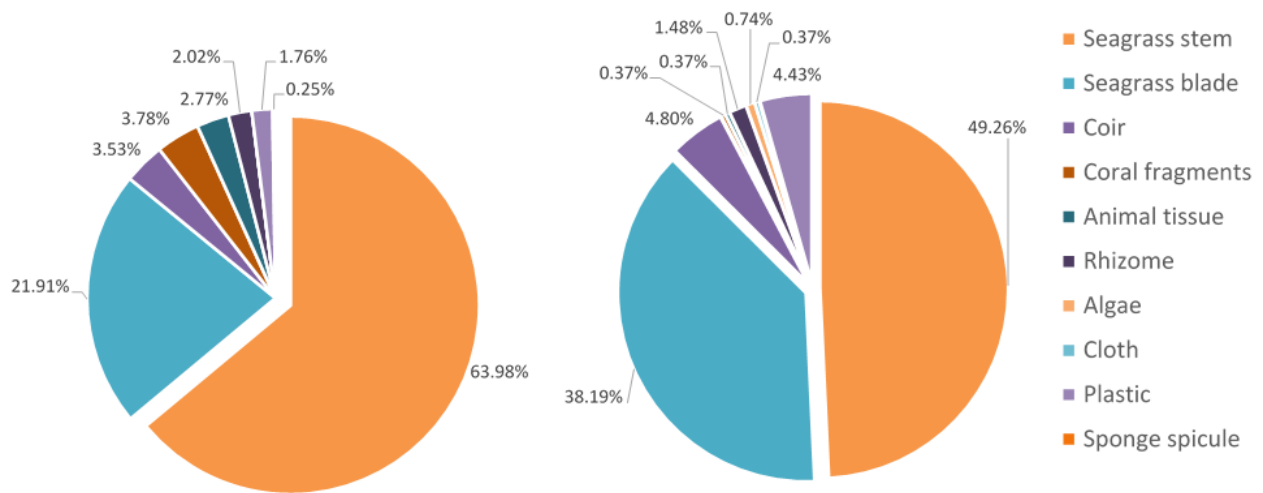


Figure 2. Constituents of green turtle feces show a clear preference toward seagrass than towards algae in Agatti Island (left side) and Kalpeni Island (right side).

as well as conservation strategies for seagrass communities. Therefore, a fecal analysis was conducted to identify components that constitute green turtle diet in the Lakshadweep islands.

A total of 39 fecal samples were collected opportunistically from the islands of Agatti (n = 18) and Kalpeni (n = 21) over 2 field seasons (2018-2019). The availability of fecal samples corresponded with the presence of turtles in the lagoons of these two islands. The feces were mainly collected when washed up on

the beaches or when floating on water. Upon collection, all samples were sun-dried for 24 hours to prevent fungal formation. The feces were then brought to the lab to separate and identify individual components. In order to separate the fecal matter, the samples were immersed in water overnight to loosen the contents. To ensure uniformity in the size of the samples, bigger samples were cut into smaller parts of which one part was used for identification (approx. 8 g). From each of the samples, macroscopic parts were separated

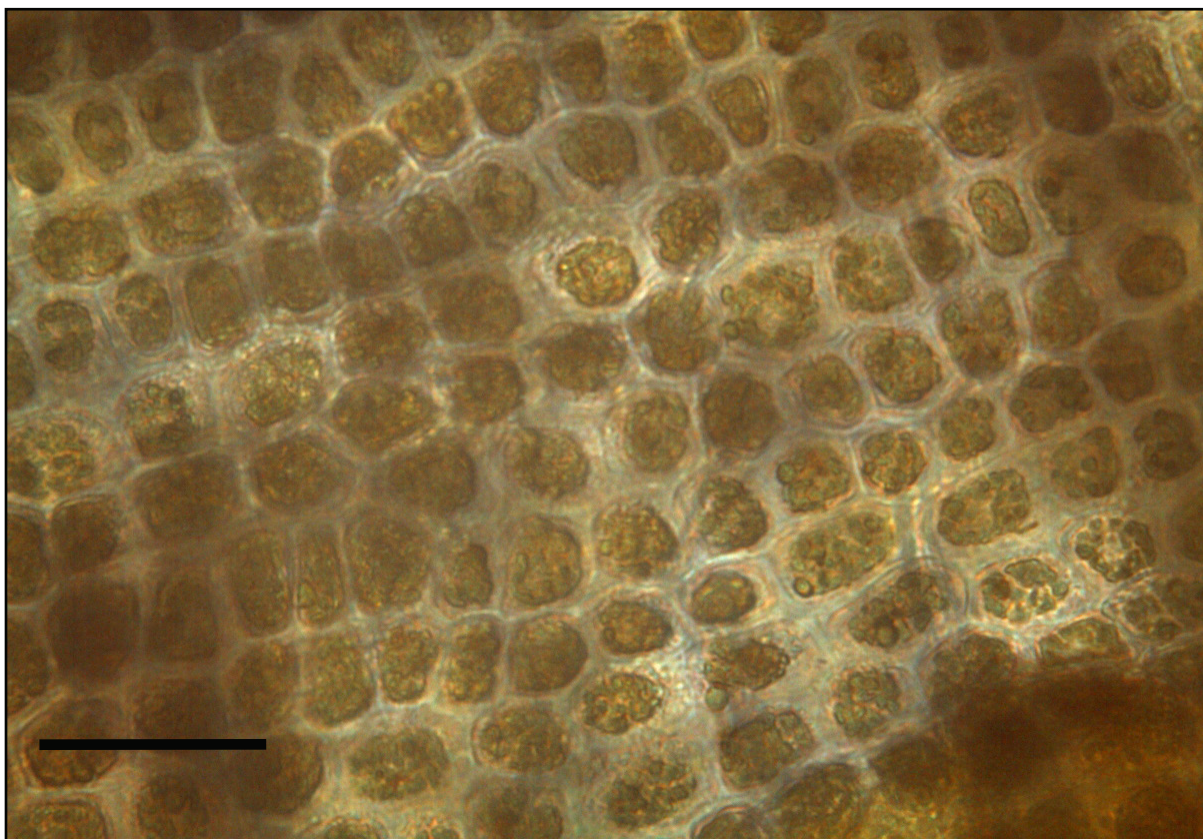


Figure 3. *Thalassia hemprichii* leaf blade found in some samples in 2018. Bar = 0.4mm.

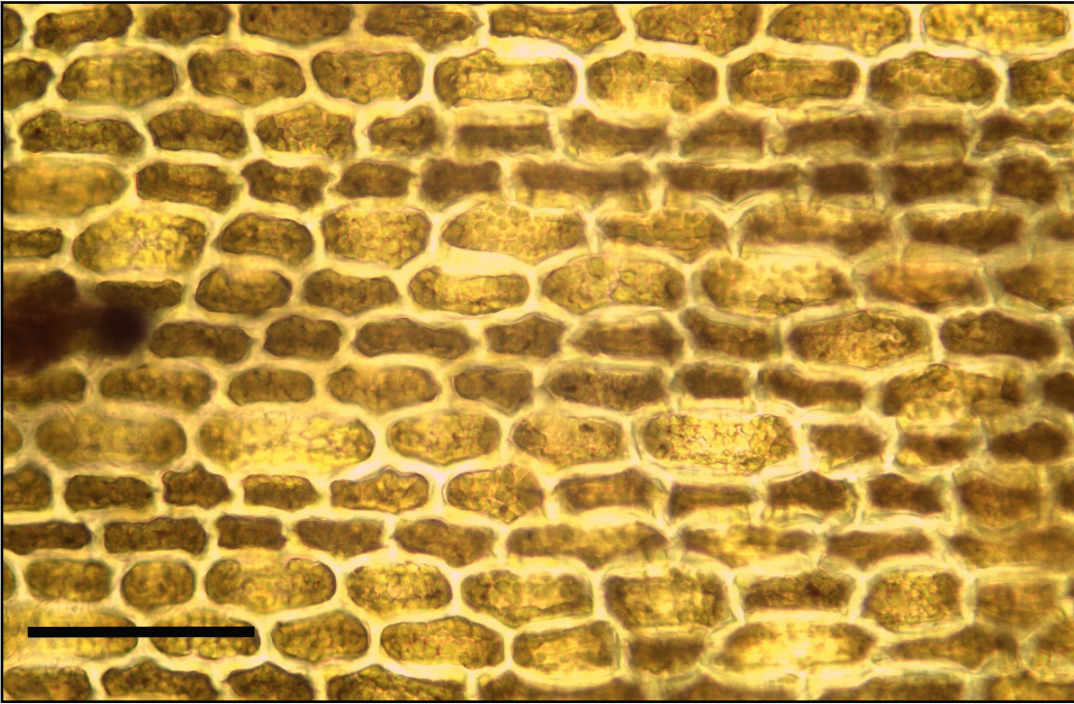


Figure 4. *Halodule uninervis* leaf blade found in two samples from Agatti. Bar = 0.4mm.

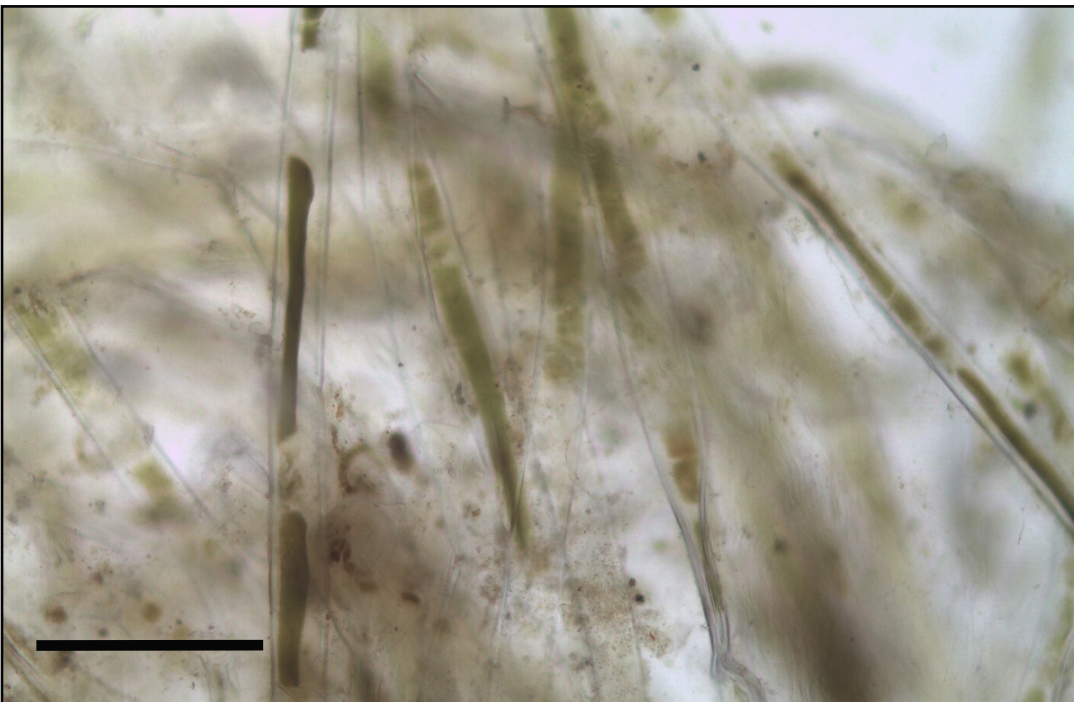


Figure 5. Filamentous green algae samples found in feces collected in Kalpeni. Bar = 0.4mm.

and stored in individual jars for identification. Identification was done using a Leica[®] microscope (Model No: DM 1000) under 10X and 40X magnification for smaller components. Each component was identified and its occurrence in the samples was recorded.

Based on these data, the frequency of occurrence of each component was calculated for different years (Table 1). Coral fragments, animal tissue and seagrass blades were more frequently observed in Agatti, but sample sizes were too small to make inferences on food choice between sites. Seagrass stem and blades formed the bulk of identified components (85-88%) of the total in both islands (Fig. 2). From the seagrass components, only the blades were useful in species identification. The species of seagrass

blades found in the feces comprised *Thalassia hemprichii* (Fig. 3), *Cymodocea rotundata* and *Halodule uninervis* (Fig. 4). Species-level identification could not be conducted using the stem epidermis or rhizomes. Coir or coconut husk was another commonly found component in the fecal matter. In addition, smaller percentages of cloth pieces, bits of plastic and small fragments of dead corals were found in some of the samples. We also found evidence of sponges in the form of spicules in 2018 and algae in 2019; however, these remain unidentified.

Our results support previous findings that the seagrass species preferred by green turtles in the Lakshadweep islands are *Thalassia hemprichii* and *Cymodocea rotundata*. In addition to

Components	Agatti (%FO)	Kalpeni (%FO)
Seagrass stem	88.8	80.9
Seagrass blade	83.3	42.8
Coir	50.0	47.6
Coral fragments	55.5	9.5
Plastic strands	27.7	57.1
Rhizome	38.8	38.1
Animal tissue	50.0	9.5
Algae	--	19.0
Cloth	--	9.5
Sponge spicule	5.5	--
<i>Sample size</i>	<i>18</i>	<i>21</i>

Table 1. Frequency of occurrence (%FO) for items - food and non-food - in green turtle feces from 39 samples.

these two species, undigested bits of *Halodule uninervis* (Fig. 3) blades in fecal samples were also detected. However, it is not clear if *Halodule* was ingested accidentally as it closely resembles blades of two preferred species or if the blades were consumed as an alternative food source. Despite the reduction in the density of *Thalassia* and *Cymodocea*, there is no clear indication that green turtles are altering their diet to compensate for the lack of resources. In addition, there was evidence of seagrass rhizomes from the samples, which shows that green turtles resort to the uprooting of rhizomes for consumption. This could prove detrimental to the recovery of seagrass if there are no remnants of the plant from which the shoots can regrow (Christianen *et al.* 2014).

Coir was also found in the turtle feces. This conforms to local observations of green turtles eating coconuts that get washed into the lagoon (Kale pers. obs. 2018). In addition, cloth pieces and plastic strands were also found in the feces. Cloth pieces are an indication of poor waste management as a lot of household waste gets thrown onto the beach, which then enters the lagoon. The presence of plastic probably indicates accidental ingestion by green turtles in the Lakshadweep islands; this coincides with global observations of increased plastic presence in sea turtle guts, even from remote oceanic islands (McCauley & Bjorndal 1999; Parker *et al.* 2011; Ng *et al.* 2016). In the Lakshadweep islands, this could be due to plastic getting trapped in seagrasses and algae or direct consumption of plastics.

Filamentous green algae were also observed in the feces from one of the two islands that were sampled, potentially belonging to *Cladophora* spp (Fig. 5). Previous studies in these islands suggest that green turtle herbivory results in a drastic reduction in densities of *Thalassia hemprichii* and *Cymodocea rotundata* (Lal *et al.* 2010; Kelkar *et al.* 2013). This study provides the first observation that green turtles may also consume algae with seagrass. This could potentially mean that, in cases of low availability of seagrass, some individuals could shift their diet toward algae. Components such as traces of sponge spicules, other animal tissue, and algal matter were observed in the fecal samples for the first time, which could

suggest that adult green turtles might have a broader diet spectrum.

Fecal analysis is not the most effective method to determine diet components at a species level as completely digested material will not be identifiable in the feces. Moreover, microscopic components such as algae, seagrass seeds, etc. cannot be easily detected using this method. Techniques such as stable isotope analysis or gastric lavage will improve our understanding of green turtle diets in this region. Broadly though, the results suggest that green turtles in the Lakshadweep lagoons are maintaining their preferred diet of seagrass species and are perhaps compensating for the lack of it by eating other seagrass species and algae. While the presence of *Halodule* sp. and algae was in very small quantities, it could indicate the onset of diet change, given the declines in overall seagrass cover and density. Further information on their diets will be useful in informing management plans for green turtles as well as for the conservation of their foraging habitats.

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First Report of a Haemosporid Parasite in a Sea Turtle

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The coccidian parasite, *Haemoproteus* sp. (Haemospororida: Haemoproteidae), sometimes called “*Hemoproteus*,” occurs in the blood of birds, turtles, tortoises, lizards, snakes and frogs around the world (Lainson & Naiff 1998), but is not known from sea turtles. This report of *Haemoproteus* sp. is presented to alert others to search for this parasite in sea turtles.

During the rehabilitation process at the University of Puerto Rico Department of Marine Sciences Sea Turtle Rehabilitation Facility (STRF) of a stranded olive ridley turtle, *Lepidochelys olivacea*, found 3 km off the northwest coast of Puerto Rico between Aguadilla and Rincon on 30 August 1997, a blood sample was taken for a red blood cell count and other diagnostics. A *Haemoproteus* sp. was found (Fig. 1). The exact abundance of this organism could not be determined, but it appeared to be present in relatively low numbers. The protozoan was identified to genus and line drawings were made. However, additional blood samples were not taken due to the weak condition of the turtle. Routine diagnostic blood samples taken after rehabilitation were negative for the protozoan. The turtle was deemed successfully rehabilitated after 162 days, and released on 06 February 1998, 60 km south of St. Croix, USVI. Unfortunately, the original slides containing the protozoan were lost in the mail before they could be deposited in a museum. The macrogametocyte (n=3, all poorly stained) was sausage shaped with a finely granular cytoplasm. It extended approximately two-thirds the way around the host nucleus displacing the nucleus only slightly (NDR [nucleus displacement ratio] = 0.5-0.6).

The border of the parasite was well defined. The parasite contained a few small, clear, intracytoplasmic vacuoles. Larger vacuoles were not observed. The cytoplasm contained 11 to 15 medium-sized, diffusely scattered, dark brown pigment granules. The parasite was

closely adhered to the host cell nucleus and occupied most of the space in the cell on one side. It was 17.2 µm long and 3.2 µm wide. The parasite nucleus (n=1, visible) was oblong, 2.2 µm long and 1.8 µm wide, and centrally located.

No infections with 2 macrogametocytes per erythrocyte were observed, but only a total of three infected cells were observed. No microgametocytes or developing macrogametocytes were found.

The genus *Haemoproteus* can be diagnosed because of its large, pigmented, halter-shaped (or sausage-shaped) macrogametocyte in the erythrocytes of hosts. It often partially or completely encircles and displaces the nucleus of mature erythrocytes (Fig. 1). The protozoan parasite described above has not previously been reported from sea turtles and probably represents a new species. However, we had too few specimens to justify description of a species.

Members of this genus are more commonly found in birds, but occasionally occur in reptiles. Most of the hosts have been terrestrial or freshwater; however, a few marine records exist (Work & Rameyer 1995). They usually cause little harm to their hosts, but debilitated hosts are sometimes killed. The extremely emaciated olive ridley turtle may have been affected by this parasite; however, the parasite appeared to have remained at relatively low numbers and did not appear to be taking advantage of any reduced immunity/resistance of the host.

Protozoan parasites of sea turtles have received relatively little attention. Herbst & Jacobson (1995) noted amebiasis, *Entamoeba invadens*, in captive sea turtles; and Márquez (1990) an amoeba, *Entamoeba* sp., in the leatherback turtle, *Dermochelys coriacea*. Graczyk *et al.* (1997) found Crypto, *Cryptosporidium* sp. infections in green turtles, *Chelonia mydas*, in Hawaii and Mercer *et al.* (2012) found it in a loggerhead turtle stranded in the Northern Adriatic Sea. A coccidian, *Caryospora cheloniae*, caused considerable disease damage in cultured green turtles in the Cayman Islands (Rebell *et al.* 1975; Leibovitz *et al.* 1978), and in free-living green turtles in Australia (Gordan *et al.* 1993). Another coccidian, *Eimeria caretta*, has been described from the loggerhead turtle, *Caretta caretta* (Upton *et al.* 1990). Chauvier (1986) found a “hémocytozoaire” parasite in the hawksbill turtle, *Eretmochelys imbricata*, in Europe. This could possibly represent the same parasite that we found, or a closely related species. Eiras *et al.* (2000) found intererythrocytic inclusion bodies in the loggerhead turtle from Madeira, Portugal. These probably represented viral, or rickettsial damage, not a protozoan.

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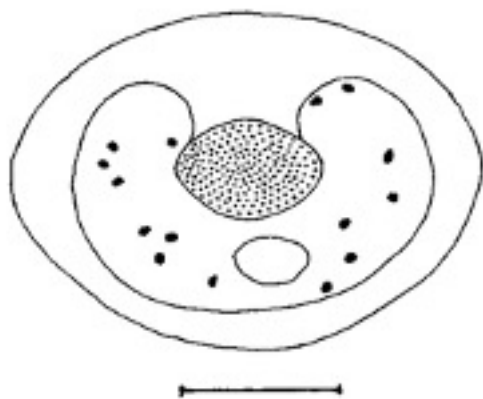


Figure 1. Macrogametocyte of *Haemoproteus* sp. in erythrocyte (red blood cell) of an olive ridley turtle, *Lepidochelys olivacea* (Chelonia: Chelonidae), found 3 km off the northwest coast of Puerto Rico between Aguadilla and Rincon 30 August 1997. Scale bar = 5 micrometers.

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Lepidochelys olivacea in Puerto Rico: Occurrence and Confirmed Nesting

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Lepidochelys olivacea (Eschscholtz 1829) is known as the olive ridley turtle, or “golfina” in Spanish, and belongs to one of the six genera of the family Cheloniidae (Reichart 1993). This species is considered the most abundant marine turtle in the world with a near-circumtropical distribution (Reichart 1993). According to the most recent update from the IUCN Red List (2008), the species has been decreasing worldwide and it is listed as a Vulnerable Species (www.redlist.org). Despite the population declines, positive trends in abundance in individual nesting sites within regional management units have been observed in the Western Atlantic and Northeast Indian Ocean (Mazaris *et al.* 2017). Olive ridleys are widely distributed along the Pacific coast of Central and South America, but they are not abundant in the Western Atlantic basin (Reichart 1993; Marcovaldi 2001). In the Western Atlantic, the highest nest densities have been reported from beaches in Suriname, French Guiana, and Brazil (Marcovaldi 2001; da Silva *et al.* 2007; Plot *et al.* 2012). The northernmost published observations of olive ridley turtles in the Western North Atlantic were in Floridian waters (Foley *et al.* 2003), where they occurred as strandings.

Although the occurrence of *L. olivacea* in the Greater Caribbean region is rare, there have been some sightings in Cuba, the U.S. Virgin Islands, and the Dominican Republic (Varona 1974; Bacon 1981; Carr *et al.* 1982; Fretey 1999; Moncada *et al.* 2000; Eckert & Eckert 2019). Eckert & Eckert (2019) report that all known nesting sites for *L. olivacea* around the wider Caribbean are found along the northeastern coast of South America and Trinidad. The presence of *L. olivacea* in Puerto Rico is infrequent (Dow Piniak & Eckert 2011; Eckert & Eckert 2019). The first recorded and officially published sighting of an olive ridley turtle in Puerto Rico waters was near the

San Juan harbor in 1967 (Caldwell & Erdman 1969). The specimen had a straight carapace length of 48 cm with a width of 51.5 cm; the specimen was determined to be a subadult (Reichart 1993). Rivero (1998) mentioned two other sightings of *L. olivacea* at Toa Baja in 1967 and at San Juan in 1976, all in northern Puerto Rico. In 1997, a female was found incidentally entangled in a net two miles offshore between Aguadilla and Rincón (Horta *et al.* 2000).

The coasts of Puerto Rico are used as nesting sites for three other species of marine turtles: *Dermochelys coriacea* (Vandelli 1761), *Eretmochelys imbricata* (Linnaeus 1766) and *Chelonia mydas* (Linnaeus 1758) (Eckert & Eckert 2019). Monitoring efforts by the Committee for Marine Turtle Conservation “Yo amo el Tinglar,” a local grassroots group, have documented 10 possible nesting events by *L. olivacea*. Of these 10 nests, five dead hatchlings recovered from four different nests were preserved. Based on morphological traits (color of the hatchling, number of scutes), the specimens collected from nests laid in Arecibo are considered *L. olivacea*. This study summarizes all available nest data of the olive ridley turtle in Puerto Rico between 2017-2019 and confirms the presence of *L. olivacea* through molecular and morphological analysis. In addition, this year (2020), a female *L. olivacea* was observed nesting by a local fisher on one of the beaches, where other nests have been previously reported. However, data from the hatchlings from this nest have not been analyzed nor presented in this study (Figs. 1 and 2). The impetus of presenting the data of 2017-2019 is to contribute to the knowledge of the biology and distribution of this species, in order to improve population assessments of the olive ridley turtle locally in Puerto Rico and in the Wider Caribbean.

The beaches with olive ridley nests were Playa Abacoa



Figure 1. Female *Lepidochelys olivacea* before laying a clutch in Playa Abacoa, January 2020.



Figure 2. One of the live hatchlings of *Lepidochelys olivacea* found in the nest in Playa Abacoa, March 2020.

(18.474028 °N, -66.703056 °W) and Playa Grande (18.491056 °N, -66.610472 °W) along the north coast of Arecibo, Puerto Rico. Regular monitoring for freshly laid nests on these beaches is common because two other marine turtle species, the leatherback turtle and the hawksbill turtle are present in these areas (Eckert & Eckert 2019). Because the nest patrols are normally conducted in the morning, there were no visual confirmations of a nesting *L. olivacea*, although the size and characteristics of the nesting crawl raised doubts about the identification of the species for each nest. After the nests hatched, they were evaluated following the protocol of Miller (1999) and the number of emerged hatchlings from each nest, the number of eggs, the percentage of those that hatched and the emergence success were recorded. Dead hatchlings were preserved in 95% ethanol. The morphological characters of the specimens were then measured (Table 1). Total genomic DNA was extracted from tissue taken from the carapace or posterior right flipper, using a Qiagen DNeasy 96 Blood & Tissue Kit (Qiagen, Germany) and following the manufacturer's protocol. A ~800 bp fragment of

ID	Carapace Scutes					Carapace size (mm)	
	Central	Left	Right	Left	Right	Width	Length
L	7	8	9	13	13	55	57
M1	5	6	6	-	-	43	55
M2	5	7	6	13	13	47	50
M3	7	7	7	13	13	56	64
M4	5	7	8	13	13	55	61

Table 1. Morphological characters of the carapace of each olive ridley specimen hatchling. (-): Specimen M1 had damaged marginal scutes, therefore, no measurements were recorded.

the mtDNA control region, or D-loop, was amplified with the primers H950g (5'-GTCTCGGATTTAGGGGTTT-3') and LTEi9 (5'-GAATAATCAAAAAGAGAAGG-3') (Abreu-Grobois *et al.* 2006). Amplification was carried in a MyCycler™ Thermal Cycler (BioRad), using the PCR conditions in Campista-Leon *et al.* (2019). The quality and quantity of all PCR products was estimated with 1% agarose gel electrophoresis and the NanoDrop™ spectrophotometer. The PCR products from five hatchlings were sent to the McLAB facility (San Francisco, CA, USA) for Sanger sequencing in both directions. Quality control, end trimming and sequence editing was done with CodonCode Aligner 9.0.1. No mutations were observed among the sequences of the five specimens (GenBank Accession Numbers MT501679- MT501683). Sequences were submitted with BLASTn (Altschul *et al.* 1997), in GenBank and the top hits results were recorded.

The north coast of Puerto Rico has dynamic sandy beaches with variable widths, shaped by storms, hurricanes and high wave energy (Morelock *et al.* 2000; Barreto-Orto *et al.* 2019). Turtle hatchling L came from a nest in Playa Grande, a sandy beach that is well covered by vegetation (Morelock *et al.* 2010). Specimens M3 and M4 also came from Playa Grande. The other two specimens were sampled from two nests of Playa Abacoa, located next to the river mouth of Río Grande de Arecibo. Open sandy beaches near river mouths appear to be the typical nesting beach profile for *L. olivacea* (Pritchard & Mortimer 1999).

Pritchard (1969) described the carapace of the Western Atlantic olive ridley turtles as having 5-7 central scutes. The common number of marginal scutes is 12 on the left and 12 on the right, but there are cases where the marginals scutes are 13 (Pritchard 1969). According to the description from Pritchard & Mortimer (1999), olive ridley turtles have five to nine costal scutes with asymmetrical configuration with a typical carapace length ranging from 38-50 mm. In a morphometric study by Michel-Morfin *et al.* (2001), the maximum curved carapace length (CCL) in hatchlings was 50 mm, while the maximum curved carapace width (CCW) was 52 mm.

All hatchlings collected (Table 1) exhibited the aforementioned described carapace scute characteristics; however, the length of the carapaces varied from that of the original descriptions. This could be due to slight carapace deformations associated with the nest conditions and/or the preservation process. The curved

ID	Site	Date		CS	HS	ES
		Date laid	emerged			
L	Playa Grande	1/18/2017	3/21/2017	105	95	92
M1	Playa Abacoa	1/4/2019	3/20/2019	108	67	62
M2	Playa Abacoa	1/25/2019	4/9/2019	120	83	68
M3	Playa Grande	7/6/2019	8/25/2019	89	47	39

Table 2. Data from individual confirmed *Lepidochelys olivacea* nests laid in Arecibo, Puerto Rico. CS = clutch size, HS = hatching success (%) and ES = emergence success (%).



Figure 3. Carapace from the dead female olive ridley found in Isla Cabra, San Juan, Puerto Rico. Numbers 1-7 indicate costal carapace scutes.



Figure 4. Skull from the dead female olive ridley found in Isla Cabra, San Juan, Puerto Rico.

carapace measurements and the manner in which the specimens were preserved, either with a straight carapace or curved carapace following the egg circumference, might have caused a small increase in the length of the curved carapace measure.

In the nests we examined, the hatching success ranged from 47 - 95% and the emergence success ranged from 39 - 92% (Table 2). The small number of inspected nests ($n = 4$) limits our inferences on the hatching and emergence success of *L. olivacea* in Puerto Rico, however they are similar to values reported in other studies. In the western Atlantic, the average duration of incubation ranged from 41 - 72 days and the clutch size of *L. olivacea* nests ranged between 4 and 182 eggs, with an average of 100 eggs (SD = 0.29) (da Silva *et al.* 2007). In the East Pacific, Barrientos-Muñoz *et al.* (2014) reported a range of 45 - 100% and 45.8 - 93.4% for hatching and emergence success, respectively.

Our five D-loop DNA sequences (625 bp length, after quality check and end-trimming) were identical to several publicly available sequences in GenBank, found in olive ridley turtles from the western Atlantic Ocean (*e.g.*, Genbank accession numbers FJ795429-FJ795433; Plot *et al.* 2012), the Mediterranean (*e.g.*, KP117262; Revuelta *et al.* 2015), Indian Ocean (*e.g.*, MN342239; Stelfox *et al.* 2020) and the Pacific (*e.g.*, JX454987; Duchene *et al.* 2012). Our sequences were identical (69% coverage) to haplotype F (*e.g.*, AF051773; Bowen *et al.* 1998). This haplotype is present in 94% of olive ridleys from rookeries in Suriname and Brazil, which harbor low mtDNA diversity (Bowen *et al.* 1998). Finally, although the control region sequences in Foley *et al.* (2003) were not published in a database, they reported that all three specimens found in Florida waters matched haplotype F. Haplotype F appears to be widespread in areas as geographically separated as Florida (Foley *et al.* 2003), Puerto Rico (this study), Suriname and Brazil.

On 6 October 2020, a sexually mature female *L. olivacea* was found dead on the coast of Isla Cabra, San Juan (18.4699671 °N, -66.1353916 °W). By the decomposition state of the body, we estimated that the turtle was found a week after her death. Since eggs were observed in the oviduct, we assumed that the olive ridley was disoriented by the light of a lamppost when it came out for nesting

and she then fell off a cliff. The female weighed 27.2 kg and the curved carapace length and width measurements were, respectively, 65 cm and 63 cm; a total of 7 costal carapace scutes were observed. The carapace and skull of the olive ridley turtle were preserved for evidence of this event (Figs. 3 and 4).

Until now, only three species of marine turtles have been confirmed nesting in Puerto Rico. Using morphological characteristics, descriptive data from the nests, and DNA data, we now confirm that *L. olivacea* is the fourth species of marine turtle that has used the beaches of Puerto Rico for nesting. Even though the nesting events have been sporadic in frequency and low in numbers, it is extremely important to continue monitoring and gathering data for olive ridley turtles in Puerto Rico for biodiversity and management purposes.

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Distinguishing Between Fertile and Infertile Sea Turtle Eggs

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Phillott & Godfrey (2020) have recently described the best practices and potential techniques for assessing fertility of sea turtle eggs and distinguishing between infertile eggs and those that experienced intra-oviducal or early embryonic death:

- physical examination of the egg exterior (eggshell) for the ‘white-spot’
- candling of the egg to visualize the embryo and/or extra-embryonic membranes
- physical examination of the egg contents for signs of embryonic development (e.g., embryo, blood, other tissues)
- microscopy or molecular methods to detect signs of sperm penetration of the ovum or syngamy

The decision tree and comparison table (Table 1) below complement Phillott & Godfrey (2020) and will help researchers who need to distinguish between fertile and infertile eggs. However, conclusions about infertility in a population should only be made with targeted studies utilizing rigorous sampling (see Phillott & Godfrey 2020).

Decision tree to assess egg fertility

- 1a. White spot on egg exterior within ~7 days of oviposition (reviewed by Phillott & Godfrey 2020)..... **Egg fertile**
- 1b. No white spot on egg exterior within ~7 days of oviposition **2**
- AND/OR*
- 1c. Candling reveals developing embryo and extra-embryonic membranes within ~7 days of oviposition
(reviewed by Phillott & Godfrey 2020) **Egg fertile**
- 1d. Candling does not reveal developing embryo and extra-embryonic membranes within ~7 days of oviposition **2**
- 2a. Macroscopic examination of egg contents locates an embryo, blood, or other tissue
(Ewert 1985; Miller 1985; Miller *et al.* 2017) **Egg fertile**
- 2b. Macroscopic examination of egg contents does not locate an embryo, blood, or other tissue..... **3**
- 3a. Microscopic examination (x10 power) of egg contents locates differentiated blastoderm or embryo
(Miller 1985; Birkhead *et al.* 2008; Miller *et al.* 2017) **Egg fertile**
- 3b. Microscopic examination (x10 power) of egg contents locates undifferentiated blastodisc
(Birkhead *et al.* 2008) **Egg infertile**
- 3c. Microscopic examination (x10 power) of egg contents does not locate blastoderm or blastodisc **4**
- 4a. Appropriate stains and microscopy reveal holes caused by sperm penetration of the inner perivitelline membrane (PVM),
sperm bound between the inner and outer PVMs, and/or nuclei in the blastodisc (Birkhead *et al.* 2008) **Egg fertile**
- 4b. Appropriate staining and microscopy does not reveal holes caused by sperm penetration of the inner PVM,
sperm bound between the inner and outer PVMs, and nuclei in the blastodisc..... **Egg infertile**
- 4c. DNA amplification and sequencing detect PVM-bound sperm (Croyle *et al.* 2016)..... **Egg fertile**
- 4d. DNA amplification and sequencing do not detect PVM-bound sperm **Egg infertile**

Definitions of terms used:

Amplification of DNA - creating copies of genetic material (DNA) using laboratory techniques such as quantitative polymerase chain reaction (PCR) to allow analysis (see *sequencing of DNA*, below) of even very small samples.

Blastodisc - or germinal disc; the embryo-forming portion of an egg, which sits on the outer surface of the yolk.

Blastoderm - the first layer of cells that form as the fertilized blastodisc undergoes cleavage (division of cells).

Candling - a method of observing embryonic development by shining a bright light source through the egg from behind or below to illuminate the embryo, extra-embryonic membranes, and yolk. Candles were originally used as the light source and gave the process its name; torches/flashlights are more commonly used now.

Extra-embryonic membranes - membranes formed as tissue outgrowths of the embryo itself during development; the vitelline membrane or yolk sac, allantois, amnion, and chorion.

False positive - an error in which a positive result is mistakenly generated, e.g., microbe nuclei may be stained with the dye Hoechst 33342 and incorrectly identified as sperm heads. This could lead the researcher to wrongly conclude that eggs had been fertilized. The risk of a false positive can be overcome by use of multiple techniques, examination by multiple experts, and a suitable sample size.

Intra-oviducal - occurring in the oviduct, e.g., intra-oviducal embryo mortality.

Oviposition - the process of laying eggs.

Post-oviposition - after oviposition; after eggs are laid.

Perivitelline membrane (PVM) - the inner PVM encloses the yolk and the blastodisc. Sperm penetration of the inner PVM triggers formation of the outer PVM, so that polyspermy (fertilization of an egg by multiple sperm) does not occur. Sperm heads that are in contact with, but have not penetrated, the inner PVM when the outer PVM forms can become trapped between the two membranes.

Sequencing of DNA - any of a number of laboratory techniques can be used to determine the order in which nucleic acids (adenine, guanine, thymine, and cytosine) occur within DNA; a DNA sequence can then be compared within a genetic sequence database to confirm the species.

Shell membrane - the membrane located on the inner surface of the eggshell.

Stage of development - embryo development has been described by Miller *et al.* (2017) as a series of stages (1-31). Development in stages 1-5 occurs in the oviduct, and stages 6-21 in the nest after oviposition. Illustrations of the developmental stages are available in Miller *et al.* (2017), while photographs of stages 19-31 can be seen in Bladow & Milton (2019).

Syngamy - fusion of the sperm and ovum genetic material.

White-spot - a chalk-white spot that forms at the north pole of a fertile egg within 7 days of oviposition. When they are laid, eggs are usually translucent. After oviposition, the vitelline membrane comes in contact with the shell membrane at the top of the egg and draws water from it. Dehydration of the eggshell changes its appearance from translucent to chalk-white. As the embryo develops, the white spot grows in size to encompass the entire egg within ~20 days.

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Table 1a. Comparison of techniques to assess egg fertility at or shortly after oviposition.

	Infertile egg	Fertile egg experiencing intra-oviducal embryo mortality	Viable, fertile egg
White spot on egg exterior	Will not develop	Will not develop	Will develop within ~7 d of oviposition (see Phillott & Godfrey 2020)
Embryo and/or embryonic membranes visible by candling	None present to be detected	May not be visible due to size	May not be visible until ≥ 1 d post-oviposition (Abella <i>et al.</i> 2017)
Embryo and/or embryonic membranes in egg contents	Undifferentiated blastodisc only; likely to be visible (see Ewert 1985, Birkhead <i>et al.</i> 2008); may require microscopy	Blastoderm likely to be visible (see Ewert 1985, Miller 1985, Birkhead <i>et al.</i> 2008); may require microscopy	Embryo, blood or other tissue should be visible by 4 d post-oviposition; may require microscopy (see Phillott & Godfrey 2020)
Holes in the inner perivitelline membrane	None formed to be detected	Likely to be detected with light microscopy (Birkhead <i>et al.</i> 2008)	Likely to be detected with light microscopy (Birkhead <i>et al.</i> 2008)
Sperm bound in perivitelline membranes	None present to be detected	Likely to be detected after staining with dye Hoechst 33342 and fluorescence microscopy, or DNA amplification and sequencing (Birkhead <i>et al.</i> 2008, Croyle <i>et al.</i> 2016)	Likely to be detected after staining with dye Hoechst 33342 and fluorescence microscopy, or DNA amplification and sequencing (Birkhead <i>et al.</i> 2008, Croyle <i>et al.</i> 2016)
Nuclei in the blastodisc	None formed to be detected	Likely to be detected after staining with dye Hoechst 33342 and fluorescence microscopy (Birkhead <i>et al.</i> 2008)	Likely to be detected after staining with dye Hoechst 33342 and fluorescence microscopy (Birkhead <i>et al.</i> 2008)

Table 1b. Comparison of techniques to assess egg fertility during or after the incubation period.

	Infertile egg	Fertile egg experiencing intra-oviducal embryo mortality	Fertile egg experiencing embryo mortality during incubation	Viable, fertile egg
White spot on egg exterior	Will not develop	Will not develop	Will fade within ~44 hr of embryo mortality; may not be visible (Phillott & Parmenter 2007)	Will completely encompass egg within ~20 d of oviposition (see Phillott & Godfrey 2020); shell exfoliation may be observed in the week prior to hatching (Sahoo <i>et al.</i> 2009)
Embryo and/or embryonic membranes visible by candling	None present to be detected	May be challenging to detect due to size and decomposition	May be challenging to detect with decomposition depending on stage of development	Visible
Embryo and/or embryonic membranes in egg contents	Blastodisc may be challenging to detect with decomposition	Blastoderm may be challenging to detect with decomposition	Embryo, blood or other tissue may be visible; may require careful sorting and microscopy (see Phillott & Godfrey 2020)	Development proceeds as described and illustrated in Miller <i>et al.</i> (2017)
Holes in the inner perivitelline membrane	None formed to be detected	May be detected with microscopy depending on decomposition	May be detected with microscopy depending on decomposition (Birkhead <i>et al.</i> 2008)	May be detected with light microscopy (Birkhead <i>et al.</i> 2008)
Sperm bound in perivitelline membranes	None present to be detected	May be detected by staining with dye Hoechst 33342 and fluorescence microscopy, or DNA amplification and sequencing, depending on decomposition. False positives may occur during staining and microscopy if microbes present and mistaken for sperm heads. (Birkhead <i>et al.</i> 2008, Croyle <i>et al.</i> 2016)	May be detected by staining with dye Hoechst 33342 and fluorescence microscopy, or DNA amplification and sequencing, depending on decomposition. False positives may occur during staining and microscopy if microbes present and mistaken for sperm heads. (Birkhead <i>et al.</i> 2008, Croyle <i>et al.</i> 2016)	May be detected by staining with dye Hoechst 33342 and fluorescence microscopy, or DNA amplification and sequencing, depending on decomposition. False positives may occur during staining and microscopy if microbes present and mistaken for sperm heads. (Birkhead <i>et al.</i> 2008, Croyle <i>et al.</i> 2016)
Nuclei in the blastodisc	None formed to be detected	May be detected by staining with dye Hoechst 33342 and fluorescence microscopy depending on decomposition; false positives may occur if microbes present (Birkhead <i>et al.</i> 2008)	May be detected by staining with dye Hoechst 33342 and fluorescence microscopy depending on decomposition; false positives may occur if microbes present (Birkhead <i>et al.</i> 2008)	May be detected with microscopy

First Record of a Stranded Loggerhead Turtle (*Caretta caretta*) in a Ghost Net off Penang, Malaysia

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Malaysia is located in the tropics, an ideal habitat for sea turtles. Four sea turtle species are known to nest in Malaysia: leatherback turtles (*Dermochelys coriacea*), green turtles (*Chelonia mydas*), hawksbill turtles (*Eretmochelys imbricata*), and olive ridley turtles (*Lepidochelys olivacea*; Chan 2006). A fifth species, the loggerhead turtle (*Caretta caretta*), was believed to have nested in Sarawak, Malaysia (Chuang 1961; Leh 1985), with in-water occurrences on the east coast of Peninsular Malaysia (Kobayashi *et al.* 2011). However, there have been no new records of loggerhead turtles nesting in the past few decades in Malaysia (Chan 2006). There are 31 loggerhead nesting sites within the Indian Ocean, which are grouped into four Regional Management Units (RMUs): the Northwest RMU (Arabian Sea), the Northeast RMU (Sri Lanka, Bangladesh, and Myanmar), the Southeast RMU (Western Australia), and the Southwest RMU (Africa and Madagascar; Wallace *et al.* 2010). The Pacific Ocean has 76 loggerhead nesting sites that are grouped into two RMUs: the Northern RMU (east Asia including Japan and Taiwan) and the Southern RMU (Australasia; Wallace *et al.* 2010).

The Indian and western Pacific Oceans (where several of the nesting sites in the southern Pacific Ocean RMU are) are bordered by developing nations whose main livelihoods are dependent on fisheries (Reeves *et al.* 1988; van der Elst *et al.* 2005). Both artisanal and commercial fisheries can contribute to abandoned, lost, or discarded fishing gear (hereafter “ghost nets”). Ghost nets can float in the ocean where they can entangle marine animals, with sea turtles being particularly vulnerable (Duncan *et al.* 2017). This is because ghost nets act as fish aggregators which can attract sea turtles to a potential foraging area (Carr 1987; Chanrachkij & Loog-On 2003; Stelfox *et al.* 2016). A review of the published and grey literature on the incidents of sea turtle entanglements with ghost nets found that the Indian Ocean was one of the understudied ocean basins (Stelfox *et al.* 2016).

Here, we report on the incidence of a loggerhead turtle entangled in a ghost net off the coast of Pulau Kendi, an island south of Penang Island on the western coast of Peninsular Malaysia.

Although it is possible that there were previous incidents of sea turtles entangled in ghost nets in Malaysia, this is the first reported incident of a loggerhead turtle found in the waters of Peninsular Malaysia entangled in a ghost net.

Penang Island is on the west coast of Peninsular Malaysia with the Indian Ocean and the Straits of Malacca to the west and south, respectively (Fig. 1). Its oceanography is largely driven by the monsoon. Currents flow east from the Indian Ocean into the Straits of Malacca during the southwest monsoon between May and August and reverse during the northeast monsoon between November and February (Shankar *et al.* 2002; <http://www.met.gov.my/pendidikan/cuaca/fenomenacuaca>).

On 7 September 2019, the loggerhead turtle was found with its head and front flippers entangled in a discarded trawler net by three inshore fishermen of Teluk Kumbar approximately 500 m from Pulau Kendi (5.2345 °N, 100.1915 °E, Fig. 1), south of Penang Island. The 2×3 m net had two different mesh sizes: 20 cm on one half of the net and 10 cm on the other. The fishermen cut the net to release the turtle (Fig. 2), but found it was too weak to swim and simply floated adrift. The fishermen brought the sea turtle to shore after they notified the head of the Teluk Kumbar Fishermen Unit, who later alerted the Department of Fisheries.

The sea turtle was transported to the Tunku Abdul Rahman Aquarium in Batu Maung for rehabilitation (Fig. 2). At the time of rescue, it weighed 60 kg with 82 cm curved carapace length and 75 cm curved carapace width. Its diet in the aquarium mainly consisted of mackerel. Based on morphology and genetic assessment conducted by the Fisheries Research Institute at Batu Maung, Malaysia, the sea turtle was confirmed to be a loggerhead turtle. However, analyses to trace the origin of the turtle were not conducted. It was released seven weeks later at Pasir Belanda, Penang Island, into the bay of Teluk Kumbar (Fig. 1), on 24 October 2019 after it displayed good health and was swimming actively within its holding tank (Fig. 2). This site is a known nesting area for other sea turtle species (Mohd Salleh *et al.* 2012) and was believed to be a suitable release site for this individual.

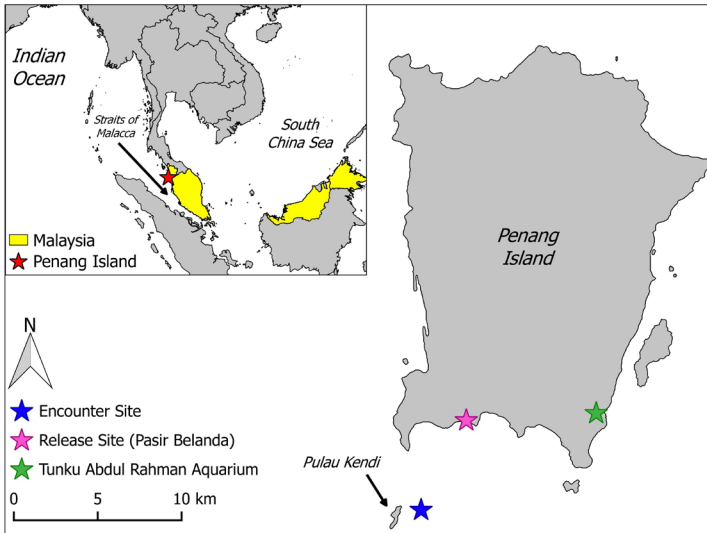


Figure 1. Location of Penang Island (red star), Malaysia (yellow polygon) relative to the Indian Ocean and the Straits of Malacca (inset), as well as the sites (stars) where the loggerhead turtle was encountered (blue), released (pink), and rehabilitated (green).

It is not known when or where the individual was entangled before it was found. The southwest and southeast Indian Ocean RMUs are a possibility due to their proximity to the Straits of Malacca (Wallace *et al.* 2010), which include Myanmar (Thorbjarnarson *et al.* 2000), the Javan Sea near the Sunda Straits (Manansang *et al.* 1995), and Western Australia (Riskas 2014). It is also possible that the loggerhead turtle may have come from the southern Pacific Ocean RMU (Wallace *et al.* 2010), such as the seas south of Sulawesi (Manansang *et al.* 1995).

The origins listed above are namely the nesting beaches for loggerhead turtles (Wallace *et al.* 2010). Because nesting beaches tend to be great distances away from foraging areas, it is likely that its foraging areas could be even closer to where this individual loggerhead turtle was found. A nesting loggerhead turtle satellite tagged in Taiwan was found to migrate to the South China Sea on the east coast of Peninsular Malaysia (Kobayashi *et al.* 2011). This points to the possibility that there could be a foraging area for loggerheads on the east coast of Peninsular Malaysia. Given that

the loggerhead turtle in Penang did not have heavy barnacle load nor was in poor body condition, there is a possibility that it may have been within an undiscovered foraging area for loggerhead turtles within the Straits of Malacca before it was entangled and encountered.

Managing ghost nets in faraway seas can be impossible, but doing so locally is easier. The origin of this loggerhead sea turtle is not known, but the possibility that it was foraging within the Straits of Malacca before it became entangled in a ghost net cannot be discounted. While the possibility of a new foraging site for loggerhead sea turtles in Malaysia can spur the proper management of ghost nets in Malaysia, ghost nets are a threat to many other marine species, and should be managed regardless. In December 2020, there was an incidence of a loggerhead turtle carcass washed ashore on Langkawi Island. This island is located to the north of Penang Island and is in close proximity to the Andaman Sea. It is possible that there is an unknown foraging ground not just within but beyond the Straits of Malacca that needs to be protected.

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Figure 2. Left panel: the loggerhead turtle was brought onto the boat and transported to shore for (center panel) rehabilitation before (right panel) being released to the sea.

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Using Social Media and Photo-Identification for Sea Turtles of New Caledonia

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In the last decade, the arrival of Web 2.0 and the rise of social media have opened a new window of opportunistic data collection. Social media is a new type of internet-based media that allows individuals to share photographs, videos, and thoughts and opinions with the world. Multiple social media platforms are available; Instagram is one of the many that allows users to document elements of their everyday lives in a visual context using a smartphone application. Instagram had over one million users within two months of its initial release and steadily increased to over 800 million users, to date (Everson 2017).

Even though most species of sea turtle are Endangered or Critically Endangered on the IUCN Red List (www.iucnredlist.org), and many questions still need to be addressed in order to implement beneficial conservation actions (Hamann *et al.* 2010), funding can be hard to obtain. Alternative methods must be developed in order to collect data for these in-water animals. Photo-identification (Photo-ID or PID) has been used widely to identify individual cetaceans and orectolobiformes (*e.g.*, whale sharks). For whale sharks, the spots on the body of individuals have been used to differentiate between individuals (Arzoumanian *et al.* 2005; Meekan *et al.* 2006; Bradshaw *et al.* 2007). Similarly, photos of cetacean dorsal fins and tail flukes have been photographed to differentiate individuals (Mizroch *et al.* 1990; Wursig & Jefferson 1990; Jujiwara & Caswell 2011; Weller *et al.* 2012). In 2008, two studies addressed the use of photo-identification as an alternative method to flipper tagging for the identification of individual sea turtles (Reisser *et al.* 2008) and Schofield *et al.* (2008) validated this technique by using individuals that also had flipper tags. Photo-identification of sea turtles has been published in multiple papers in more recent years using citizen science sightings. Home ranges of four individual turtles were calculated in Caribbean Honduras using photographs collected by citizen scientists and a smart-phone application that geo-located all uploaded photographs (Baumbach *et al.* 2019). A different study mapped the distribution of 199 individual sea turtles with the help of diver tourists at La Reunion Island (Chassagneux

et al. 2013). Citizen science (recreational scuba divers) has also been successfully used in monitoring the composition, size and distribution of sea turtles in Mozambican waters (Williams *et al.* 2015), while Papafitsoros *et al.* (in press) used photos posted on social media to document individual sea turtle behavior in Greece.

New Caledonia is a French territory located in the South Pacific (Fig. 1) that is famous for its white sand beaches as well as the world's longest continuous barrier reef. It also has six areas classified on the World Heritage List for its reef diversity and associated ecosystems (UNESCO 2008). As of 2019, New Caledonia is home to a total of 271,407 inhabitants (ISEE 2019a) and has an average of 120,000 tourists that transit through the international airport each year (ISEE 2019b). Cruise ships also provide another type of tourism in New Caledonia and these visitors spend a day independently sightseeing or joining organized day tours. As of 2019, 300 cruise ships had stopped in New Caledonia, carrying over 500,000 tourists (<https://la1ere.francetvinfo.fr/nouvellecaledonie/tourisme-croisiere-pleine-expansion-783863.html>).

Photo-identification programs have been conducted for multiple species in New Caledonia. The first study was started in 1991 to identify the migration of humpback whales (Garrigue & Gill 1994) and is still ongoing (Garrigue *et al.* 2004, Orgeret *et al.* 2014). A second study obtained sea snake sightings from a group of retirees and it was discovered that there were 140 individual snakes in a coastal area of the capital of New Caledonia (Goiran & Shine 2019). Although these studies conducted were PID, they were done without the use of automated software. A study using photo-identification and the software FinBase reported the migration of tiger sharks in the waters south of New Caledonia (Clua *et al.* 2013). In this study, we documented photographs of sea turtles foraging in New Caledonia that were posted on Instagram between 2013 and 2019 and analyzed them using a program called TORSOOI (Jean *et al.* 2010). We found photographs on Instagram through a search for the keywords: #seaturtle in three languages (French, English and Japanese), #newcaledonia, popular diving sites of New Caledonia

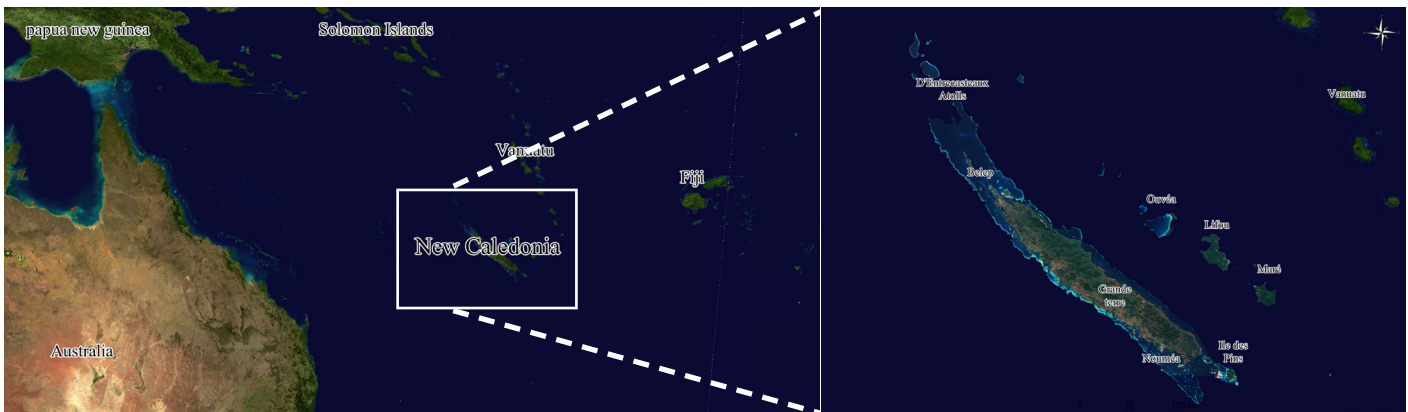


Figure 1. Map of New Caledonia (DTSI-Gouv NC).

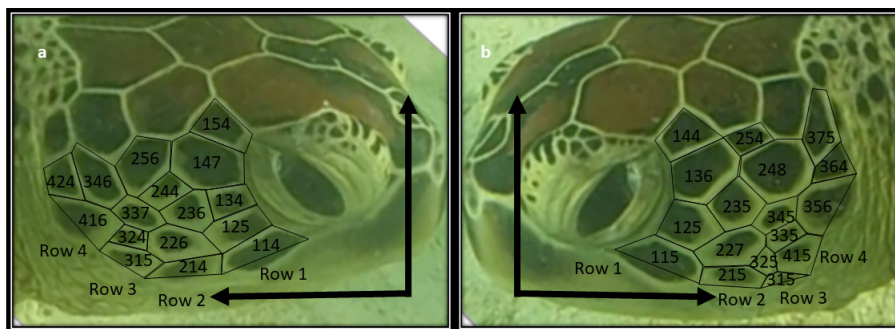


Figure 2. Viewpoint for scute analysis of an individual green turtle’s right facial profile (a) and left facial profile (b), based on the position and the shape of the scutes.

Photo ID-Code IM10 right:

114-125-134-147-154-214-226-236-244-256-315-324-337-346-416-424

Photo ID-Code IM10 left:

115-125-136-144-215-227-235-248-254-315-325-335-345-356-364-375-415

#noumea, #amedeeisland, #maitreisland and words such as #barrierreef, #lagoon, #dive, and #underwaterphotography.

Once photographs were collected, we analyzed the profiles for individual turtles using the photo-ID software TORSOOI with all the metadata related to the sighting (date, place where the photograph was taken, species, life-stage, sex, particular physical characteristics such as missing limbs, etc.). The first step consists of manually drawing outlines of the scales on the facial profile by clicking on the vertices around the scutes. This automatically generates a series of codes (one for each scale), which are then used in the recognition process to order match results (Fig. 2). These codes indicate the position of the scale on the profile: first digit corresponds to the row, second digit to the line and the third digit equals the number of sides that each scute has. These scales have been studied and remain stable throughout the life of the individual (Carpentier *et al.* 2016). Match results are ordered according to the number of matched codes to photos (Jean *et al.* 2010). Finally, we visually matched photographs from the query. If a match was validated, the history of the profile was completed: the photograph was added to the record belonging to the matched profile (with all the metadata associated) and recorded as a “recapture.” This allows us to monitor the movements of individuals and to note how many times an individual was seen. We recorded any turtle as a new individual if the photograph did not have a match in the database. When the right

and left profiles of the same turtle were available, they were linked and the record for that individual was complete.

Our search revealed that 946 sea turtle photographs were uploaded to Instagram between 2013 and 2019 and were referenced as having been taken in New Caledonia. Forty-six percent (n = 437) of these photographs were useable on the program TORSOOI (Fig. 3). We uploaded each of the useable photographs to TORSOOI and identified 276 profiles. Three species of sea turtles were photographed: *Chelonia mydas* (90%), *Caretta caretta* (5%) and *Eretmochelys imbricata* (5%). Our data showed *C. mydas* as the dominant species in New Caledonian waters; this was previously reported by Read (2015). Fourteen different locations within New Caledonia were included in the photograph metadata. They varied from the whole territory to specific islands (Table 1).

We found TORSOOI recognized profiles that already had matches in our database, and these profiles were then recorded as “recaptures.” In this study, 164 recaptures were found with each profile being sighted on average 1.8 ± 1.4 (SD) times (range of recaptures per profile: 1-9) and with a mean of 277 ± 346 (SD) days between each recapture. All recaptures were of *C. mydas* profiles. Recaptures were recorded in seven locations but the highest rate of recapture was at Amedee and Signal Islands. Amedee Island is the primary tourist attraction listed on Tripadvisor (tripadvisor.com) and is directly linked to cruise ship tourism, while a snorkeling company on Signal Island advertises a “Noumea turtle tour” for tourists. These tourist attractions may explain why most recaptures were recorded at these two locations. Our data show that only one individual was photographed at two different locations. A single *C. mydas* named “Flo” was photographed at Duck Island on 31 October 2017 and was subsequently photographed on 16 November 2017 at Maitre Island (Fig. 4). Other studies support our results that sea turtles show high fidelity to their feeding areas (Limpus *et al.* 1992; Musick & Limpus 1997; Chaloupka & Limpus 2005; Broderick *et al.* 2007; Limpus 2009; Hart & Fujisaki 2010; Read *et al.* 2014).

Scientific data are harder to collect for juvenile and male sea turtles as they are rarely observed. Opportunistic PID allows the gathering of information for individual foraging sea turtles that would otherwise be difficult to obtain. Previous research has shown that in New Caledonia, the majority of green turtles present in foraging grounds are juveniles (Read *et al.* 2020). Our data does not allow us to estimate the number of individuals per feeding ground, however, citizen science data collection may aid future studies in providing these types of estimates.

In this study, the photographers had no particular training and no

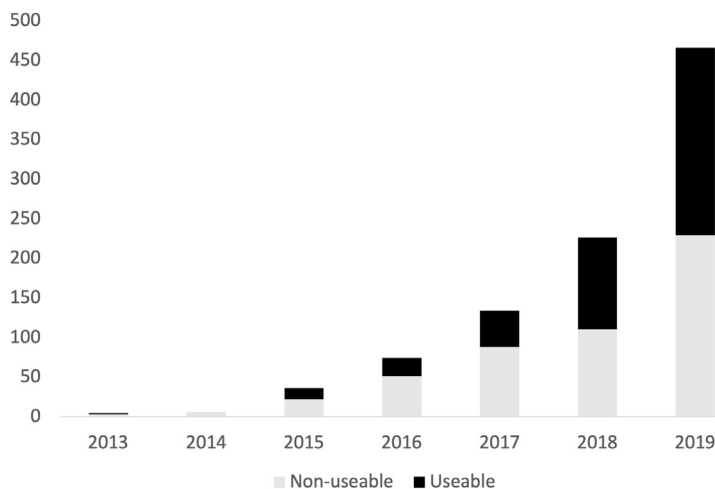


Figure 3. Number of photographs of sea turtles uploaded on Instagram between 2013 and 2019 in New Caledonia. Black bars represent useable photos, whereas grey bars represent non-useable photos.

New Caledonia	Noumea	Poé	Koumac	Lifou	Mare	Ouvéa	Ile des Pins	Duck Isld	Maitre Isld	Signal Isld	Laregnere Isld	Amedee Isld	Tenia Isld
48	6	2	2	10	3	6	13	7	58	25	6	85	5

Table 1. Number of useable sea turtle photographs that were uploaded to Instagram and their geographic locations.



Figure 4. Map of islands of Noumea (DTSI-Gouv NC).

knowledge of participating in a citizen science project. The citizen scientists that provide the best data are the ones that are trained over time; this has been demonstrated in several studies (Williams *et al.* 2015; Baumbach *et al.* 2019). By also giving guidelines to people who want to participate in PID studies, it may also prevent some natural behavior disruption or harassment of the sea turtles (or other species studied). We witnessed instances of this disruption in some of the photographs from Instagram. In the Maldives, a citizen science study has allowed for data to be collected by dive and snorkel guides, marine biologists, and tourists (Hudgins *et al.* 2017). Their study was able to show that a citizen science project is an effective way of collecting data and that it also helps engagement of the general public toward the conservation of endangered species (Hudgins *et al.* 2017). Scuba divers represent an adequate population to participate in citizen science but there is a drawback of a lack of long-term involvement (Lucrezi *et al.* 2018). A previous study conducted by Santori *et al.* (2020) had observed this kind of behavior with users of TurtleSAT, a program designed to record any sightings of freshwater turtles in Australia. Their study showed direct contact with volunteers is needed and may be implemented through a hotline for technical issues (Santori *et al.* 2020).

The majority of the photos of sea turtles that were uploaded only showed one side of the head of the individuals. The number of scales and their disposition changes from one side to the other, which represents a bias in the data since the right facial profile is different from the left facial profile (as per Fig. 2). Therefore, individuals may be counted twice if the facial profile photographs cannot be

matched to other photographs in the database. The use of videos or guidelines for citizens willing to provide data is essential in order to get a complete individual (Newman *et al.* 2010).

Not all photographs uploaded on social medias are useable (distance, angle, photo quality, etc.). Creating a hashtag dedicated to your study and giving some guidelines into what type of photographs is needed will allow for a non-negligible gain of time and of data. However, launching a campaign to collect some data can have some negative impact on the natural behavior of sea turtles. The race for the perfect picture can sometimes impede the precautions usually taken to protect these endangered species that may also be against the law in some countries. For example, in New Caledonia, approaching sea turtles at a distance <10 m is against the law.

The impact of whale watching on the behavior of humpback whales was studied in New Caledonia, in which 80% of the individuals showed a clear deviation from their trajectory when boats approached (Schaffar *et al.* 2013). A similar study was done on sea turtles in Honduras and showed that the number of sightings did not vary with the pressure from divers but the time spent feeding, investigating and breathing decreased with the presence of divers (Hayes *et al.* 2017).

This study describes an innovative method for data collection and explores the quality of citizen science photos for use in PID. This type of project could be undertaken in other areas where tourists are in close proximity to sea turtles, but rules should be put in place to make sure that sea turtles are not negatively impacted more than what is gained for their survival.

- Acknowledgements.** The photographs used for this study follow the fair use provision, Section 107 of the U.S. Copyright Act. Only a subset of the photographs was used and the data corresponding to the photographers were not stored. A special thank you to the volunteers who spent hours looking for photographs on social media for this project.
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