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Male hawksbill turtle emergence just above the surf zone, on Cousine Island, Seychelles. See pages 9-10.
Photo by Nina Voogt.

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Successful Use of Buccal Swabs to Obtain Genetic Material from Loggerhead Sea Turtles (*Caretta caretta*)

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Traditionally, the use of genetic material from sea turtles has been acquired from adult individuals employing invasive techniques like taking blood or skin tissue to obtain DNA. These methods tend to be expensive and time consuming and may harm or stress the individuals sampled, particularly neonates and juveniles because blood is usually drawn from the dorsal cervical sinus in the neck or from the femoral venous plexus in the hind flipper (Wyneken 2001; Wallace & George 2007), while skin is sampled using a razor blade or biopsy punch to collect tissue from the top few layers of the turtle's epidermis (Dutton & Balazs 1995; Dutton 1996). Both methods, particularly blood sampling, require sample-handling protocols that may not be feasible under challenging field conditions or they may not be permitted by regulatory agencies. It is often necessary to hire a specialized technician as it takes expert skills to take the sample, as well as prophylactic processes required to decrease the risk of infection. Biological samples obtained from juvenile loggerhead turtles (*Caretta caretta*) have been used to isolate DNA employing traditional protocols, which has resulted in several common difficulties such as creating wounds and increasing stress levels. Here we propose the use of buccal swabs as an alternative method to avoid these complications on these relatively fragile life stages (Deelman *et al.* 2002; Freeman *et al.* 2003); this method is based on briefly scraping epidermal cells from the mouth, providing sufficient amounts of tissue to extract DNA for genetic studies. This technique has been used for reptiles such as tuataras, *Sphenodon punctatus* (Miller 2006), newts, *Triturus alpestris*, and frogs, *Hyla arborea* (Broquet *et al.* 2007). However, in collecting DNA for endangered species such as marine turtles it has had limited implementation (Lanci *et al.* 2012).

The buccal swab method is an easy way to obtain biological samples from sea turtles of any size and life stage that would prevent damage to individuals. This is a low-cost method that can be applied to multiple individuals in a relatively short time when gravid females or neonates aggregate along the beach or are individually captured in the sea. The information obtained from genetic analyses constitutes one of the most relevant lines of evidence to understand levels of organization and variability of populations (Marco *et al.* 2009). The identification of haplotypes is useful in conservation management, particularly since sea turtles have been exploited around the world during recent decades with resulting population declines. Low population recruitment results in an imbalance in population dynamics and decreased survival rates in the population increases the risk of extinction (Margalef 1977; Eckert 2002). The loggerhead turtle is currently considered Endangered (EN) internationally (www.redlist.org) and Critically

Endangered ("Peligro crítico") in Colombia (Castaño-Mora 2002). Therefore, it is important to study population structure in terms of variability, heterozygosity and allelic diversity as part of conservation efforts to protect loggerheads in different habitats and along migration routes.

The 20 loggerhead samples were obtained from head-started juveniles at the Sea Turtle and Marine Mammals Conservation Program (ProCTMM) of the Jorge Tadeo Lozano University research group "Dinámica y Manejo de Ecosistemas Marinos y Costeros (DIMARCO)," category A-COLCIENCIAS. Dermal epithelial cells were taken while the mouth was carefully kept open using cables and hoses for better handling and with a sterile cotton applicator scraping was performed on the tongue and on the palate for approximately 5 seconds. This was done with the endorsement of the Corporación Autónoma Regional del Magdalena-Corpamag, through cooperation agreement N° 04 de 2011. The research permit was granted by DADMA resolution No. 081 of 5 April 2011; these organizations are local and regional environmental authorities, respectively.

DNA was isolated from 20 buccal swabs collected from juveniles (approximately 3 months in age, average carapace length = 15 cm). Different sterilized cotton swabs were used for each turtle to avoid contamination during the buccal swab sampling process. Each sample was immersed in 500 µL of lysis buffer (SDS 2%), followed by proteinase K (Bioline) digestion for 16 h at 65°C. Samples were cooled to -4 °C for 30 min, DNA precipitation was done with 500 µL of 5 M NaCl and centrifuged at 13,000 RPM for 30 min at 4 °C. 500 µL of supernatant was mixed with 500 µL of -4 °C isopropyl alcohol. Samples were kept at this temperature for 16 h. The samples were centrifuged at 13,000 RPM for 20 min to purify and isolate genetic material, and then the aqueous phase was discarded. 500 µL of 70% ethanol was added to wash out impurities and the sample was centrifuged at 13,000 RPM for 20 min at -4 °C. Ethanol was carefully discarded and the remaining alcohol was dried on paper towels with the sample tubes upside down for 3 h. Finally, 40 µL of TE buffer was added to preserve the DNA. An electrophoresis gel was carried out on a 1.3% agarose gel to confirm DNA isolation (Fig. 1).

To assess DNA quantity and quality, 1 µL of each DNA sample was added to a Nanodrop® ND-2000 Spectrophotometer (Thermo Scientific). Absorbance was measured at wavelengths of 260 nm and 280 nm, and relative absorbance ratios (260/280 nm and 260/230 nm) were calculated to evaluate the DNA purity obtained from the buccal samples.

PCR amplification (Saiki *et al.* 1988) was carried out for 20 buccal swab samples, using primers designed by Norman *et al.* (1994).

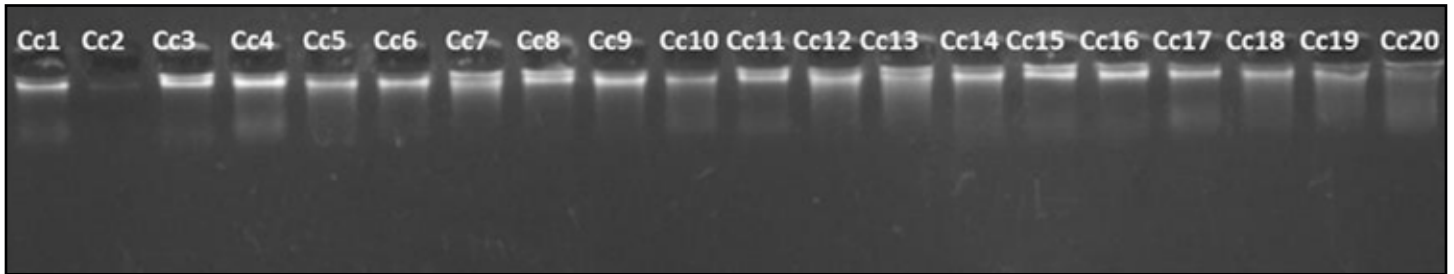


Figure 1. DNA agarose gel electrophoresis of 20 loggerhead DNA samples isolated from buccal swabs.

We amplified a 380-bp D-loop mitochondrial control region with the primers TCR5 TTGTAGATCTACTTATTTAGCAC and TCR6 GTAGGTAGAAGTAAAAGTAGGGTATGGC. PCR products were checked on a 2.3% agarose gel (Fig. 2) and sequenced using the Sanger method (Macrogen). Sequences were edited, aligned and compared with available sequences from BLAST databases using Geneious software (version 8.0.3, <http://www.geneious.com>; Kearsse *et al.* 2012).

Genetic material isolated from buccal swabs of 20 *C. caretta* individuals had high DNA quality and quantity, low degradation and low quantities of RNA (Fig. 1). The samples had 260/280 ratios of <2.0 (mean = 1.87). The 260/230 ratios were higher with range of 0-3.84 with a mean \pm SD of 1.24 \pm 2.43. As a point of reference, we compared our results with those done by Lanci *et al.* (2012); we found similar results and concentrations.

All sequences identified corresponded to known *C. caretta* haplotypes. After maximum likelihood analyses of the 15 sequences, three haplotypes (CC-A1, CC-A2 and CC-A17) were found from the nesting sea turtle population from Don Diego beach (Santa Marta, Colombia). With the BLAST search, we found these haplotypes from Green Cape in Cape Verde and in Brazil nesting sea turtles (CC-A.1 and CC-A.17 haplotypes); these locations have major aggregations of nesting *C. caretta*. DNA haplotypes from our specimens correspond to relatively well-known feeding areas (CC-A.1 and CC-A.2 haplotypes) (Garofalo *et al.* 2013), and migration routes (haplotype CC-A.2) (LaCasella *et al.* 2013). Haplotypes CC-A1 and CC-A2 are ancestral and likely originate in lineages from Atlantic and Mediterranean Sea populations. The presence of these haplotypes among the Western Atlantic Ocean, the Mediterranean Sea, and Indian Ocean basins may suggest a sequence of colonization as these haplotypes occur regularly in

these regions (Franco 2010). Haplotype CC-A17.1, from Cape Verde (Africa), is a foraging area, and the second oldest known colony in the Atlantic (Shamblin *et al.* 2014). These haplotypes are shared throughout the North Atlantic and the Mediterranean; the North Atlantic gyre circulation pattern likely helps the colonization of new beaches and foraging areas.

The data we obtained here reveal the genetic potential in terms of variability for *C. caretta* in Colombia. It is urgent to carry on genetic studies including analyses for longer sequences (800 bp), with the aim of clarifying the structure and genetic relationship of Colombian loggerheads with global populations. We conclude that the buccal swab method of tissue collection is effective, easy, fast, and an economic alternative to obtain good quality and quantity DNA. This non-invasive technique is advantageous over invasive procedures, with possibly less damage to an individual. Also, this method can provide similar results in less time with fewer resources, thereby providing scientific contributions for future research. Nevertheless, the use of blood and skin samples allows for replication of the process and the storage of subsamples for further study. Using the swabs, this is more difficult, because extracting the DNA for a second time is not guaranteed because most of the cells are used in the initial extraction.

Our genetic results indicate that Colombia turtles share relationships with global aggregations, bringing awareness that Colombia is an important habitat for turtles; protection and conservation of sea turtles there must be a priority. This points out the urgent need for increased research efforts and protection of marine ecosystems such as nesting habitat, with the aim of controlling and reduce numerous anthropogenic threats and deterioration that may affect the permanence of these populations.

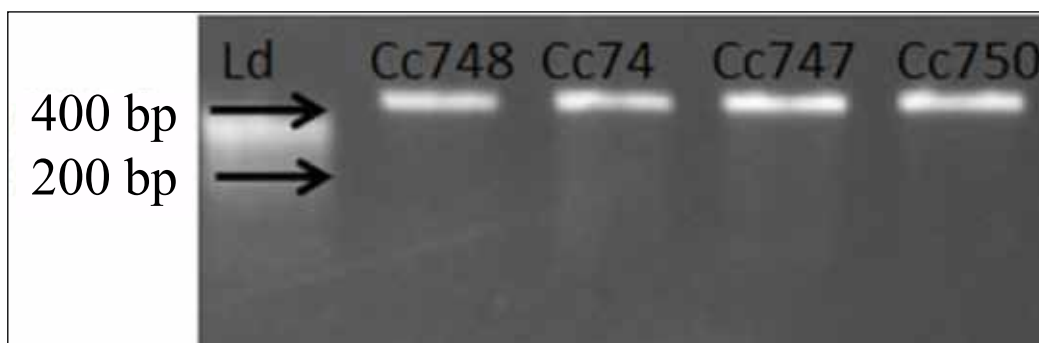


Figure 2. Electrophoresis of PCR products, amplicon of 388 bp corresponding to the control region of the mtDNA of loggerheads. Ld = ladder.

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A Leatherback Sea Turtle Entangled in Fishing Net in Mersin Bay, Mediterranean Sea, Turkey

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Three species of marine turtles, the loggerhead *Caretta caretta*, the green turtle *Chelonia mydas* and the leatherback *Dermochelys coriacea*, are found regularly in the Mediterranean (Godley *et al.* 1998; Margaritoulis 2003; Rees *et al.* 2004). Loggerhead and green turtles nest in the eastern Mediterranean, while leatherbacks are considered migrants from the wider Atlantic (Godley *et al.* 1998; Margaritoulis 2003; Casale *et al.* 2003).

Casale *et al.* (2003) reported a total of 411 records of leatherback turtles for the whole of the Mediterranean. Rees *et al.* (2004) reported a live leatherback turtle in the coast of Syria. From the coast of Israel, one leatherback was captured incidentally in a trawler net, and subsequently died from debris ingestion (Levy *et al.* 2005) and another stranded dead entangled with a static long-line (Levy 2010). Bearzi *et al.* (2015) reported a leatherback in the semi-enclosed Gulf of Corinth, Greece. Türkozan & Kaska (2010) summarized records for this species from the Aegean and Mediterranean coasts of Turkey, including a dead leatherback found stranded on the shore of the Anamur-Bozyazı Highway in southeastern Turkey (for details, see Taşkavak & Farkas 1998). Here we report on another leatherback entangled in fishing nets on the coast of Mersin.

On 11 May 2016, a leatherback was observed in Mersin Bay, about 3 m from the shoreline on Adnan Menderes Avenue, Mersin (36.7766 °N, 34.5837 °E). The leatherback's head, front right and left flippers were entangled in a piece of net that appeared to have been clipped from a larger net, presumably by a fisherman. The leatherback was observed passively floating at the surface of the water, entangled in the piece of net. One of the authors (AHU) swam to the turtle and cut the net with clippers, in order to free the head and right front flipper. Subsequently, the turtle was observed to breathe and use its flippers. Soon after, the turtle dove beneath the surface of the water before the net attached to the left front flipper could be freed. The turtle appeared to swim away easily, and it was assumed that the remaining net would fall off eventually. The turtle was not measured and sex was not verified. The total length of the turtle was less than the height of the researcher who freed it (180 cm). The turtle was photographed but it is not possible to discern

tail length (Fig. 1). We recommend that efforts should be made to include the tail in photographs of future incidents of stranded turtles, to allow sex to be assigned.



Figure 1. Floating leatherback, entangled in fishing net, off Adnan Menderes Avenue, in Mersin Bay, Turkey, on 11 May 2016.

	Location	Date	Type of encounter	Condition	Sex	Reference
Aegean coast of Turkey	Edremit Bay, near Ören, Balıkesir province	1997-10-05	Entangled in gill nets set in 10-13 m water	Dead	A large subadult, male	Baran <i>et al.</i> 1998; Taşkavak <i>et al.</i> 1998
	İzmir Bay, between Foça and Karaburun, İzmir province	Last week November 1997	Captured in trammel net, brought ashore, released back to sea	Alive	Unknown	Taşkavak & Farkas 1998
	Coast of İzmir Bay, İzmir province	2011-10-28	Stranded on the coast; two deep transversal cuts on carapace	Dead	Mature, female	Taşkavak <i>et al.</i> 2015
Southern coast of Turkey	Antalya province	1985	Captured by fishermen and brought to the harbor of Antalya	Unknown	Unknown	Baran & Kasperek 1989
	Strait of Hurma, Karataş, Adana province	1994	Photographed by officials of the Ministry of Agriculture and Rural Services, Karataş District Directorate, as it landed on the shore dead in the Strait of Hurma	Dead	Adult, unknown sex	Oruç <i>et al.</i> 1997; Oruç 2001; Türkozan & Kaska 2010
	Karataş, Adana province	1995	Found dead in nets by the fishermen of Karataş	Dead	Unknown	Oruç <i>et al.</i> 1997; Oruç 2001
	Coastline near the Anamur-Bozyazı Highway, Mersin province	1998	Stranded between rocks on the shore	Dead	Unknown	Taşkavak & Farkas 1998
	Beach in İskenderun Bay, İskenderun, Hatay province	2006-06-06	Stranded dead on the beach; originally tagged in 2005 while nesting at Matura beach, Trinidad	Dead	Adult female	Sönmez <i>et al.</i> 2008.
	In Mersin Bay, off Adnan Menderes Ave., Mersin, Mersin province	2016-05-11	Observed about 3 m offshore, entangled in fishing nets	Alive	Unknown	This study

Table 1. Reports of the leatherback turtle (*Dermochelys coriacea*), coasts of Turkey.

Local fishermen report that they occasionally encounter leatherbacks (Oruç 2001). To date, there are three records of leatherbacks from the Aegean coasts of Turkey (Taşkavak *et al.* 1998; Taşkavak & Farkas 1998; Taşkavak *et al.* 2015) and five records of leatherback from southern coasts of Turkey (Baran & Kasperek 1989; Oruç *et al.* 1997; Oruç 2001; Taşkavak & Farkas 1998; Sönmez *et al.* 2008) (Table 1). This new record of the leatherback turtle is reported from the coast of Mersin, Turkey, increasing the total number of records in southern coasts of Turkey to six and the total number of records in coasts of Turkey to nine.

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Long Distance Movement Between Nesting Sites for Two Green Turtles in the Eastern Mediterranean

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The green sea turtle (*Chelonia mydas* L. 1758) has a complex life history that is difficult to study directly. Much of what is known about life history of green turtles has come from studies focused at nesting beaches (Bowen *et al.* 1992). Hatchlings and juveniles move among several habitats during development, and adults migrate between foraging and nesting grounds (Carr 1980). During a single nesting season, an adult female typically lays two to seven clutches, each consisting around 100+ eggs. Hatchlings emerge after incubation and enter an oceanic habitat where they may remain for several years until the juvenile period (Carr 1987). Juveniles eventually recruit to neritic foraging habitats and switch to a primarily herbivorous adult diet (Pritchard 1976).

There are two important hypotheses about the philopatric behavior of female green turtles, who are observed to return to the same nesting sites year after year. The “natal homing” hypothesis states that females return to the same nesting region where they had hatched, to nest in successive seasons (Carr & Orgen 1960; Carr 1967). The hypothesis has been most extensively developed for green turtles. Another hypothesis, proposed by Hendrickson (1958) and Owens *et al.* (1982), is the “social facilitation” hypothesis and suggests that first-time nesting females follow older, experienced females to a nesting beach. Once the neophyte adult females successfully nest, they will return to that site for future egg deposition. Historically, these hypotheses were difficult to test directly, in part because of the logistical challenge of applying tags to hatchlings that would be observed on the nesting beach when they return as adults. Tagging data of nesting females can be informative of female nest site choice (Pritchard 1976), although neophyte nesting females cannot be tagged until they have already chosen a nesting beach, and incomplete monitoring effort for tagged females using nesting beaches at night remains a logistical challenge.

Natal homing should result in demographically distinct assemblages of nesting females, because successive generations of daughters should return to the same beach to nest. In contrast, social facilitation should result in high rates of female-mediated gene flow across nesting beaches (Bowen *et al.* 1992). Overall, analyses of mitochondrial DNA, which is maternally inherited, indicate that assemblages of nesting females are demographically isolated, which supports natal homing (Lohmann *et al.* 2013). Nevertheless, there remains some question as to how faithful individual sea turtles are to specific nesting beaches. Here we present records of two green sea turtle adults tagged in two different nesting beaches, that were observed nesting elsewhere in the same nesting season.

The first record comes from a green sea turtle observed laying eggs during the 2006 nesting season on both Akyatan and Yumurtalık beaches. A metal tag (ID: TR-504) was placed on the left front flipper during nesting at Akyatan beach (36.6228 °N, 35.1900 °E)

in late June. She measured 95 cm curved carapace length (CCL), 85 cm curved carapace width (CCW), 88.5 cm straight carapace length (SCL), 69 cm straight carapace width (SCW) and weighed 95 kg. This same turtle was seen at Yumurtalık beach (36.6952 °N, 35.6928 °E) while laying eggs in mid-July, by the local field team. These two nesting sites are more than 70 km distance from each other coastally.

The second record comes from a green sea turtle that nested both in Syria and Turkey during the summer of 2011. On 21 June 2011, a green turtle with a plastic tag on its left front flipper (ID: 09/34562) was observed nesting south of Latakia, Syria, on Snoubar beach (35.4370 °N, 35.8984 °E). On 15 July 2011, this same female was observed nesting on Samandağ beach (36.1174 °N, 35.9235 °E). The turtle’s measurements were CCL=92 cm, CCW=80 cm, SCL=87 cm, and SCW=68 cm. These two beaches are approximately 100 km apart from each other coastally.

Many sea turtle workers interpret natal homing to mean that females faithfully return to the beaches where they were produced. However, both examples from the individuals given above moved between beaches. Miller (1997) stated that a breeding female turtle will likely place her nests in relatively close proximity (0 to 5 km), although a small percentage utilize more distant nesting sites in the general area. Green turtles are reported to show a high degree of nest site fidelity, such as on Tromelin Island in the Indian Ocean, where most re-nesting attempts were within 200 m of the previous attempt, with a range of about 600 m (Bosc & Le Gall 1986). Similarly, Hendrickson (1958) found on islands in Malaya and Sarawak that tagged green turtles returned repeatedly to the same beach to nest, although 3-7% failed to return to the same island previously used, and these islands were about 500 m apart. The approximate curved line distances between Snoubar-Samandağ and Akyatan-Yumurtalık beaches are 100 km and 70 km, respectively (Fig. 1). Bjørndal *et al.* (1983) reported the distance between re-nesting attempts of loggerhead turtles ranged up to 290 km, but the proportion of individuals that moved hundreds of km is low. Margaritoulis (1998) reported that nine individual loggerhead turtles failed to return to the same beach previously used and one of them changed twice. The shortest distances between nests laid in different sites by individual turtles ranged from 85 to 365 km (Margaritoulis, 1998). More recently, Ehrhart *et al.* (2014) reported two loggerhead turtles were observed nesting on beaches in the SE USA that were nearly 700 km straight line distance apart. Thus, while many sea turtles may show strong nest fidelity, some may show reduced nest fidelity. Margaritoulis (1998) provided two possible reasons for this observed behavior in some loggerhead turtles. The first is that “disturbances at the nesting site can make an individual turtle change its general nesting area” and the second is that the “individuals that changed

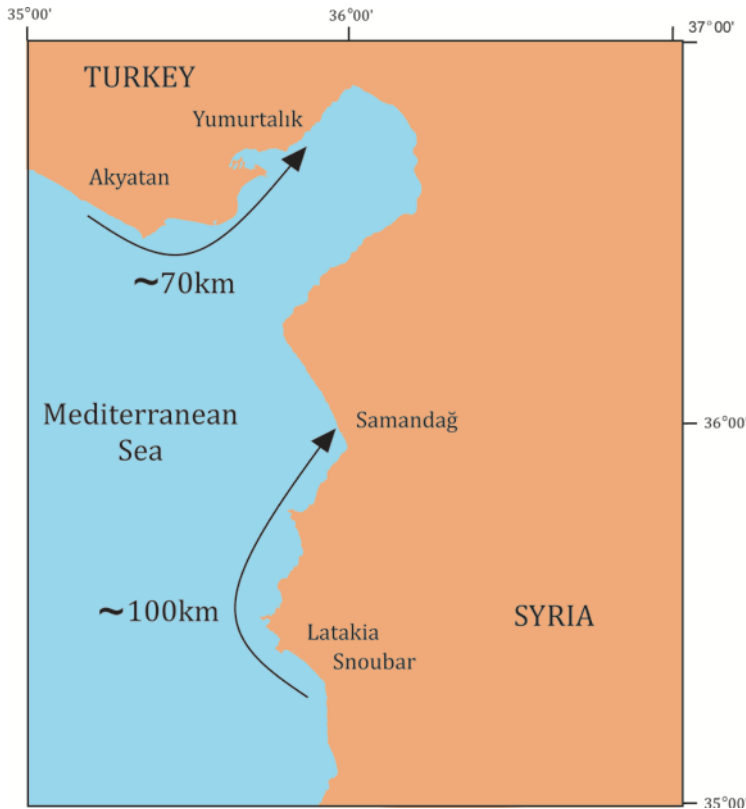


Figure 1. Locations of two different green turtle females that nested on different beaches in the eastern Mediterranean in a single nesting season.

their nesting area, were somehow lost or confused.” We add another possible reason: these turtles are opportunistic and will nest in an appropriate nesting area that is available or accessible to them.

Overall, these two records further illustrate that some breeding female green turtles do nest on different beaches, even in the same nesting season, and it is likely “the nesting population” should include adjacent beaches or regions, to account for the longer distances between nests of some individual females in the population.

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Terrestrial Emergence Recorded for a Male Hawksbill (*Eretmochelys imbricata*) on Cousine Island, Seychelles

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Basking behavior (sea or land based) is one of the less understood behaviors in marine turtles. Land-basking is a widely documented behavior for green turtles (*Chelonia mydas*) in Hawaii and the Galápagos (Whittow & Balazs 1982; Murakawa & Balazs 2005; Van Houtan *et al.* 2015). Possible reasons for land-basking include predator avoidance (Balazs 1980), energy conservation (Balazs 1980; Garnett *et al.* 1985), thermoregulation (Hughes 1974; Van Houtan *et al.* 2015), and females escaping unwanted attention from males (Booth & Peters 1972; Hughes 1974; Mortimer 1981). It has been suggested that the occasional beached male in the Galápagos occurs incidentally due to following females ashore (Green 1997). At-sea basking has been recorded in olive ridleys (*Lepidochelys olivacea*) (Hughes & Richard 1974) and loggerheads (*Caretta caretta*) (Green 1997). Murakawa & Balazs (2005) compiled a bibliography of all known terrestrial basking and non-nesting emergence publications for marine turtles, but there is no reference to basking behavior by hawksbills (*Eretmochelys imbricata*).

Cousine Island (-4.350577 °S, 55.647527 °E) is a privately owned island, which features a resort and a nature reserve in the

inner-granitic Seychelles. Its one kilometer stretch of beach is a nesting beach for both the diurnally nesting hawksbills (September - March) and the nocturnally nesting greens (rare, with one or two females observed nesting each year). The Cousine Island Marine Turtle Research and Monitoring Program began in the early 1990s, and during the hawksbill nesting season, hourly beach patrols are performed, tagging and reading tags of turtles that come ashore, and recording and monitoring nests and hatchlings. During the nesting season there are a few males observed lingering on the surrounding reef.

On 21 September 2016, a hawksbill was observed on the beach at 17:00 h, exiting the wave wash. Initially, from a distance, the turtle was perceived to be a female emerging from the surf and coming ashore to nest. When the turtle did not continue crawling up the beach, as a female would do during exploration or nesting, we became concerned and moved to another vantage point to take a better look at the turtle. We viewed the turtle from the side, noticed an unusually long tail, and then realized it was a male. He had crawled just past the surf (Fig. 1) and showed very little movement,



Figure 1. Returning to the sea after being tagged. Note the characteristic elongated tail of the male hawksbill turtle.

only twice lifting his head to make a gulping motion. Occasionally he would crawl forward if the waves washed over him. Once the sun began setting, he returned to the sea at 18:00 h. He was then caught in the shallows and brought back onshore whereupon we read his flipper tags and checked for injuries or signs of sickness. The external examination revealed no evidence of trauma, lesions, growths or ectoparasites, with the exception of a few small barnacles on the soft tissue of the hind flippers. He was very strong and healthy. The left flipper tag read SEY 6229 (on the second scale) while the right tag was missing. There was evidence of a flipper tag scar on the right second scale, therefore, the tag was presumed to have been pulled out. A replacement tag (SCA 7677), was placed in the skin adjacent to the first scale on the right flipper.

From Cousine Island internal records, this turtle was originally tagged SEY 6229/6228 (second scales) and identified as a male on Cousine Island by P.M. Hitchins in October 2003. The record showed that the turtle was observed during a similar time of the year, with the same duration out of the water, and on the same area of beach as sighted in 2016. This rare case was published as a photo of “basking” and the “only record of a male emerging onto the beach in the Seychelles” in a book describing Cousine Island’s restoration and conservation programs (Samway *et al.* 2010).

These two terrestrial emergences by this one individual male are fascinating, and if these two incidents on Cousine Island were truly land-basking, they were incredibly rare occurrences. This is the first known recorded hawksbill male to demonstrate this behavior in the Seychelles.

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Exceptional Leatherback Turtle Stranding Event in the Moroccan Atlantic During 2015

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Leatherback turtles (*Dermochelys coriacea*) have been recorded throughout the Mediterranean Sea but are concentrated in certain areas, including the Tyrrhenian and Aegean Seas, and the area around the Sicily strait (Margaritoulis 1986; Casale *et al.* 2003). The absence of nesting sites (Groombridge 1990; Laurent *et al.* 1999) and the absence of small juveniles, which are restricted to tropical waters (Eckert 2002), suggest that leatherback turtles observed in the Mediterranean are of Atlantic origin. They reach the Mediterranean Sea through the strait of Gibraltar in small groups (De Los Rios Y Los Huertos & Ocaña 2009; Casale & Margaritoulis 2010). Leatherbacks are known to occur along the Mediterranean Moroccan coast (northwest African continent), from Strait of Gibraltar east to the border with Algeria (Casale *et al.* 2003; Rojo-Nieto *et al.* 2011).

Leatherback turtles are seldom observed along the Moroccan Atlantic coast; only stranded sub-adult and adult individuals have been observed and no nesting has been confirmed there (Tiwarei *et al.* 2001; Casale & Margaritoulis 2010). Along the coastline of Casablanca, rare cases have been recorded in the past by the National Institute of Fisheries Research (INRH). During 2015, we documented 10 stranded leatherback turtles, and we conducted a survey along the shoreline of Casablanca to make sure that all stranding events were recorded. We synthesise here the information of these stranding cases, as well as available historical data on leatherback turtle stranding events along the entire Atlantic and Mediterranean Moroccan coasts, with complementary data from southern peninsular Spain and the Canary Islands.

The INRH is the Moroccan government authority that manages data on stranded sea turtles and provides scientific advice in managing stranding events. For all reported stranding events, we recorded standardized stranding information, including species identification, location, and biometric and biological parameters, and we collect tissue samples, primarily skin and muscle. We obtained data for 2015 from field operations for the Casablanca region, complemented with data from the Royal Gendarmerie of Morocco,

who respond to stranding events throughout the Moroccan coast. Historical data for Morocco were extracted from a reconstructed stranding database (Masski & De Stéphanis 2015). Data for Spain (1980 to 2012) were gathered from an online biodiversity database (<http://siare.herpetologica.es/>). For all databases, images were used when available to collect supplementary information and measurements using ImageJ (Schneider *et al.* 2012).

We conducted a beach survey on foot along 105 km of shoreline around Casablanca, to search for stranded sea turtles, and to interview local coastal inhabitants. The people we spoke to were asked if they could identify leatherback turtles on photographs and they were questioned on how frequently they observed this species during their lives. The 22 respondents, ranging in age from 19 to 56 years old, had all participated in fishing activities for fish, shellfish or urchins.

Between 14 November and 12 December 2015, we observed five stranded dead leatherback sea turtles along 25 km of the coastline in the area of Casablanca (Fig. 1). The stranded individuals were all female and had a mean curved carapace length (CCL) of 130 cm ± 18 SD (range: 118 - 162 cm). Two individuals were in early stages of decomposition and the three others were freshly stranded. None of them was thin or showed injuries or signs of interaction with fishing gear and one of them seemed to have arrived alive on the sandy beach. Two of them were necropsied and no anomalies were observed in the lung, liver, heart or kidney. The intestines were full of digesta, but the single stomach that was analysed was empty. Furthermore, no obstructing material (*e.g.*, plastic bags) was observed in the trachea after dissection. On 23 January 2016, a decomposing individual with 120 cm CCL was observed stranded in the same area. Its death may have occurred in December 2015.

Earlier during the 2015, five other stranded leatherback turtles were recorded in a 120 km area around Casablanca in February, May, July, September and November. The first turtle was decomposed and had lost its internal organs (98 cm CCL), while the others were between 103-144 cm CCL and were in the first stages of decomposition. One of them was female, and the others were not examined due to being buried before the INRH was alerted.

The survey conducted around Casablanca did not lead to the discovery of any other stranded leatherback sea turtles. During the interviews, only two fishermen recognised the leatherback sea turtle. One of these fishermen described a stranding a few months earlier and the other

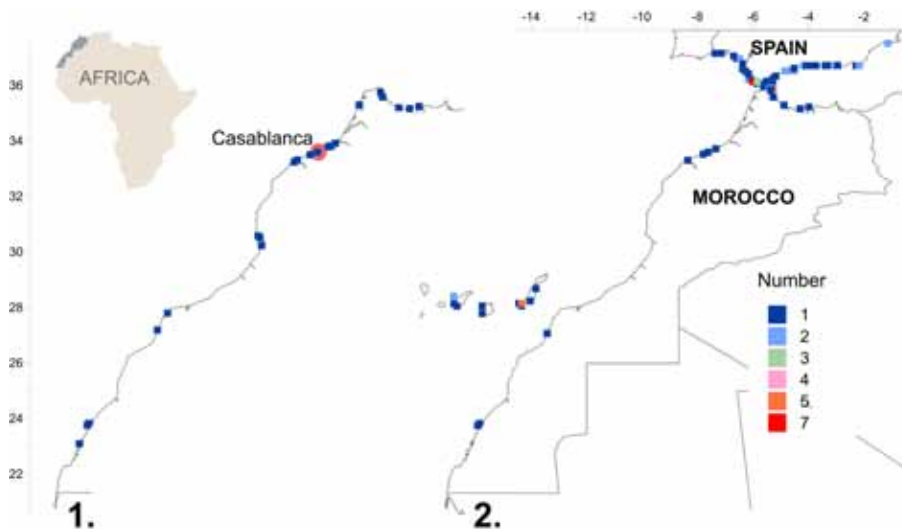


Figure 1. Leatherback sea turtle strandings 1. In the Moroccan coast for 2015, 2. historical records in Morocco (1990-2014) and Spain (1980-2012 from: <http://siare.herpetologica.es/>).

fisherman recounted that a large turtle had been caught 15 years ago by a fishing boat. The lack of recognition of the leatherback turtle by the local population supports the apparent scarcity of stranded leatherbacks in this area.

The review of historical leatherback stranding records prior to 2015 showed that four out of 21 records occurred in the Casablanca area and dated back to December 1990, July and December 2004, and December 2005. Most stranding events were recorded in the last two decades, which may reflect increased survey effort of the least surveyed areas (Casale *et al.* 2003). Four cases were recorded in the Saharan area, a coastline of more than 1000 km long where the detection probability of stranded turtles is low. The fourteen other cases occurred in the Mediterranean, of which 10 were recorded in three weeks in October and November 1995 on 8 km of coast on the Mediterranean coast close to the strait of Gibraltar.

Although rare, the leatherback turtle appears to be present all months of the year in Moroccan waters, as indicated by stranding events. This is concordant with what is observed in the Alboran sea (Rojo-Nieto *et al.* 2011), Portuguese waters (Nicolau *et al.* 2016) and in the Bay of Biscay (Morinière & Dell'Amico 2011). Few leatherback stranding events were documented along the entire Moroccan coast, which may be due in part to underreporting. The shoreline of Casablanca is a highly populated region and stranding events are nearly always reported by local and law enforcement authorities. The multiple reports of stranded leatherbacks during 2015 in this area were unusual. Two mass stranding events occurred in winter in the western Mediterranean coast of Morocco in 2004 and during the 1980s (De Los Rios Y Los Huertos & Ocaña 2009), close to the strait of Gibraltar. These events probably occurred during the migration of leatherbacks into the Mediterranean of groups of sub-adults and adults, but females seem to be more common. Wind and current patterns influence where dead or debilitated sea turtles end up on land (Hart *et al.* 2006), and stranding probability decreases with increased distance from the shore (near zero for >100 km). The Casablanca coastline receives yearly stranded cetaceans (Masski & De Stéphanis 2015) and 2015 was not different in this regard from other years, suggesting that there were no exceptional environmental circumstances that led to the leatherback stranding event. We suspect that the death of these observed stranded leatherback turtles in 2015 may have occurred in an area close to Casablanca. The stranded leatherback turtles did not have signs of interaction with fishing gear and none of the other data collected pointed to a specific cause of the stranding events. Indeed, the cause(s) of stranded leatherback sea turtles remain to a large extent unknown (Nicolau *et al.* 2016). More consistent and standardized reporting of stranded sea turtles in Morocco may help better characterize the occurrence of leatherbacks in Moroccan waters, and the threats they face.

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ANNOUNCEMENT

A Sea Turtle Nesting Beach Indicator Tool to Help Identify Areas with Potential for Sea Turtle Nesting

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In many countries where sea turtle nesting occurs or is expected, there is a paucity of data for understanding the spatial extent of nesting and relative habitat value of different beaches. As sea turtle nesting areas are a commonly encountered issue for development in some regions, and given the conservation importance of the species affected, we seek to promote enhanced detection of marine turtle nesting beaches in the absence of prior biological data. A better understanding of the likelihood of sea turtle nesting will allow the management of impacts and risks associated with projects.

The low level of baseline understanding can be due to lack of funding to deliver research, lack of easy accessibility to nesting beaches, focus on more easily accessible areas that skews spatial data, the lack of in-country expertise to undertake detailed research or the vast extent of beaches that need to be surveyed in areas where turtle nesting may occur during relatively short seasonal periods. There can also be a tendency for long term research to focus effort in areas that are known to support nesting, have historically been thought to be of greater importance and/or form part of protected areas.

We have developed an electronic tool to assess the nesting potential of beaches at minimal cost. The simple approach of the tool is seasonally independent. The approach should facilitate

information to be collected rapidly across areas that are currently poorly understood, perhaps using local networks that do not require specialist knowledge – thus helping to quickly improve the understanding of nesting potential across wider areas. This may then act as a steer for the prioritisation of funds to promote research into understanding areas that are identified as being of greatest interest; and help to create a rapid assessment of nesting beach value across regions in a way that has not yet been achieved. This could then feedback into the creation of new data sets, which if made accessible, should help to promote better developer decision-making. It should be noted that the tool deals with physical habitat features only and that it provides *indicative* rather than conclusive results on nesting potential, i.e. indications of poor suitability for certain beaches should not be used as evidence there is no nesting. The tool is useful *in lieu* of, and supplementary to, seasonal surveys that record the signs of nesting activity. In most cases the indications should be followed-up by specialist surveys.

The tool's inputs and outputs are divided into two sections (Fig. 1): the first section assesses the potential for supporting a viable nesting population, based on nearshore and beach geomorphology; and the second assesses if human disturbance is already likely to be impacting nesting activity. The tool (and accompanying explanatory documentation) is freely available, with no cost to download from www.bluedotassociates.com. It has been reviewed by several sea turtle researchers with experience from around the globe, but we are keen to hear how it can be improved and hence welcome feedback sent to thetoolbox@bluedotassociates.com.

Figure 1. The Sea Turtle Nesting Beach Indicator Tool user interface.

BOOK REVIEW

Title: Between the Tides: In Search of Sea Turtles

Year: 2012

Author: George Hughes

Publisher: Jacana Media (Pty) Ltd

ISBN: 978-1431405626

Pages: 252 (softcover)

Price: US \$23.95

As a budding environmental professional and aspiring sea turtle conservationist, I certainly consider myself passionate about sea turtles. *Of course*, I dream of saving sea turtles from the many threats they face worldwide, and I plan to work hard towards that goal in my forthcoming career. Yet how hard do scientists *really* fight in the name of understanding and conserving their species? Upon reading George Hughes' remarkable book, *Between the Tides: In Search of Sea Turtles*, I found my answer. This 250-page publication presents a collection of riveting stories about the lengths this well-known conservationist has gone to protect sea turtles in southern Africa. His commitment to the cause has taken him to far corners of the earth on thrilling, sometimes risky, journeys, sometimes requiring him to overcome immense challenges.

Nowadays, as noted by Godfrey (2005), scientific writing has become devoid of personal opinions, anecdotes, and colorful language. Hughes' book provides a refreshing exception to that pattern; he strikes a balance between both factual information and exciting stories of his many adventures in sea turtle research.

Hughes' writing captivates your attention from the very start, when he tells a particularly special tale of an elderly, decrepit female loggerhead nesting for possibly the last time in her life on the beaches of Maputaland, South Africa. He states, "Her every feature demanded respect and we somehow felt that this unique moment was to be savoured to the full" (p. 13). This moving story showcases the great admiration the author has for sea turtles, thus setting the stage for an inspiring account of his endeavors to describe their life history.

Through other lighthearted, suspenseful, and shocking stories, the author makes it clear that turtles are worth appreciating, and it quickly becomes easy for even someone who is not eco-minded to sympathize with their threats and consider the conservation implications of their actions. Never have I felt more humbled than when reading Chapter 4, *From the Beginning*, when the author describes the primitive living conditions he endured while monitoring sea turtle nests for many years on the beaches of

Maputaland. Their "modest cottage", as he calls it, lacked electricity and running water, and the author recalls regularly journeying over 15 kilometers through rugged and dangerous terrain to retrieve water that would sustain the turtle researchers while living on the beach. At one point I found myself wondering, "Why didn't they just give up?" The answer, of course, was because the turtles needed protecting, and it was clear that nothing was going to stand in the way of that. Furthermore, there seems to be a strong emotional connection between Hughes and the turtles he studies, made evident by several of his life choices, such as selecting an aragonite blue car. His justification was: "Simply because aragonite... is the primary mineral making up the shell of a sea-turtle egg and a sea-turtle egg is a very unique and valuable thing" and "rather like turtle hatchlings, I am attracted to blue things" (pp. 118).

Possibly the most commendable aspect of the book is that it frequently pays homage to the tireless efforts of dozens of sea turtle researchers and conservationists throughout the years. He also kindly acknowledges by name the many people who assisted him in his research endeavors, even those who provided him a bed on which to spend the night or a boat ride across the occasional river. Rather than seeming boastful, Hughes paints a collaborative picture of the collective determination of hundreds of scientists and volunteers to understand all aspects of sea turtle biology, ecology, and conservation.

Despite its many overwhelmingly positive characteristics, there are three shortcomings that are worth mentioning. First, *Between the Tides* appears to lack a well-organized structure, and would have benefitted from being arranged chronologically or by topic. The photographic section that bisects the book also appeared unorganized and random at times. Secondly, I would have enjoyed more information on the author's perceptions of the current and future state of sea turtle conservation within the region and beyond. For example, what are the highest-priority research considerations for the future? Finally, an index would have been helpful for the reader to return to specific sections. However, these drawbacks are minor and do not in any way undermine the importance of this work. Overall, this publication will likely be of interest to a wide range of audiences, from individuals merely looking to learn more about sea turtles, to well-established sea turtle biologists in need of a reminder why they chose their line of work.

GODFREY, M.H. 2005. Sea Turtles: A Complete Guide to Their Biology, Behavior, and Conservation. *Marine Turtle Newsletter* 108:28-29.

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RECENT PUBLICATIONS

This section is compiled by the Archie Carr Center for Sea Turtle Research (ACCSTR), University of Florida. The ACCSTR maintains the Sea Turtle On-line Bibliography: (<http://st.cits.fcla.edu/st.jsp>). It is requested that a copy of all publications (including technical reports and non-refereed journal articles) be sent to both:

The ACCSTR for inclusion in both the on-line bibliography and the MTN. Address: Archie Carr Center for Sea Turtle Research, University of Florida, PO Box 118525, Gainesville, FL 32611, USA.

The Editors of the Marine Turtle Newsletter to facilitate the transmission of information to colleagues submitting articles who may not have access to on-line literature reviewing services.

RECENT PAPERS

- AK, O., H. POLAT, E. KUCUK & H. SELEN. 2016. First confirmed record of the green sea turtle, *Chelonia mydas* (Linnaeus, 1758) (Cheloniidae), from the eastern Black Sea waters of Turkey. Turkish Journal of Zoology 40: 1-4. DOI: 10.3906/zoo-1503-7. (O. Ak, Central Fisheries Research Institute, Trabzon, Turkey).
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THESES & DISSERTATIONS

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