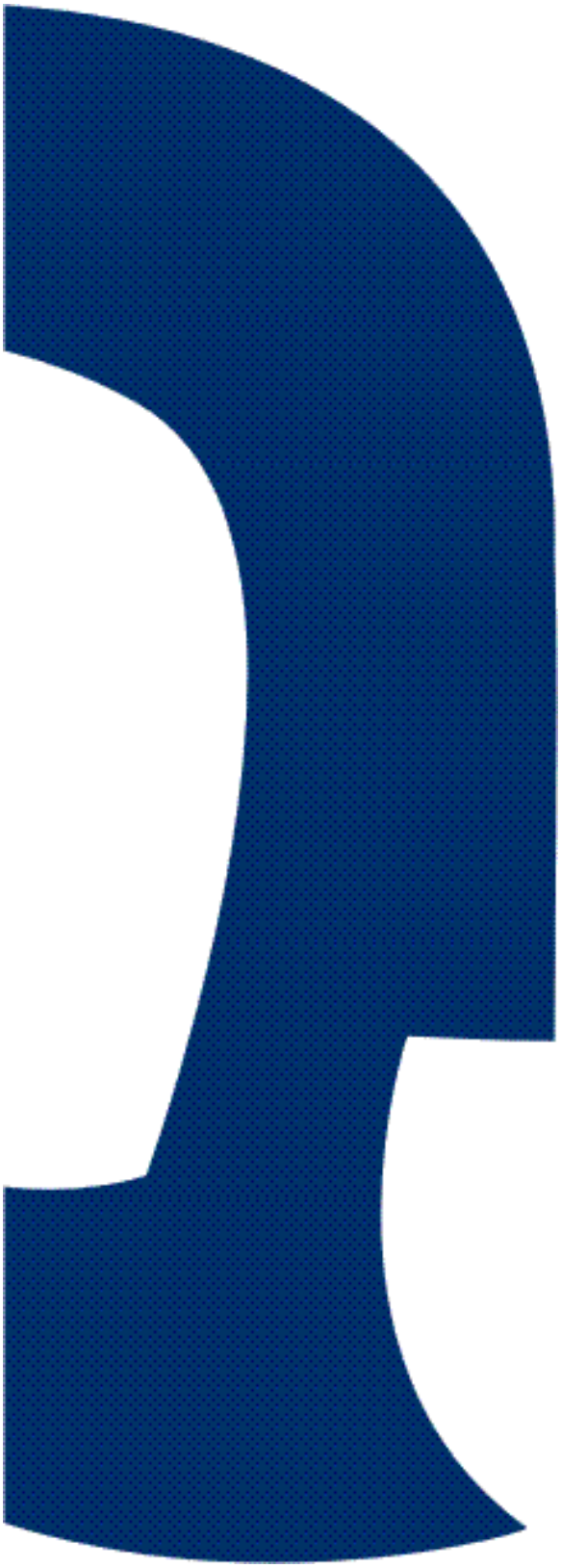

Connectivity of loggerhead sea turtle populations in Macaronesia: Genetic markers and stable isotopes

Margalida Monserrat Barceló
Master in Coastal Management
2017/2018

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Research project: Understanding connectivity between African foraging grounds exploited by the major loggerhead rookery of Western Africa.

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Master's Thesis to obtain the University Master in Coastal Management

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Abstract

Loggerhead sea turtle (*Caretta caretta*), globally threatened, is the most common sea turtle species in Macaronesia, where juveniles mix in oceanic foraging grounds around Azores, Madeira and the Canary Islands. Furthermore, the second largest nesting aggregation of this species in the Atlantic occurs in Cabo Verde Islands. This project aims to determine the spatio-temporal connectivity of loggerheads feeding in Macaronesian waters using genetic markers and stable isotopes. With this objective, mitochondrial DNA control region sequences and stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in carapace scute layers of juvenile loggerheads stranded in the Canary Islands and adult females nesting in Cabo Verde were analysed and compared with published data from Azores. Foraging ground centric many-to-many mixed stock analysis revealed that, in the Canary Islands foraging ground, the juveniles come basically from the Northwestern Atlantic (Florida) and less than a 10% from Cabo Verde. Stable isotope ratios differentiated five isospaces: (1) Azores, (2) Oceanic Canary Islands, (3) Neritic Canary Islands, (4) Oceanic Cabo Verde and (5) Neritic Cabo Verde. The analysis of carapace scute layers of turtles of the Canary Islands revealed that 61% of the juveniles sampled presented only isotopic values of oceanic Canarian waters. The remaining 39% presented values of three other foraging grounds, including the following: oceanic Canary Islands and neritic Canary Islands (4%), Azores and oceanic Canary Islands (9%), oceanic Canary Islands and oceanic Cabo Verde (22%), oceanic Cabo Verde and neritic Canary Islands (4%). These results revealed that some individuals have changed their habitat or have been moving between habitats during their development, showing, for the first time, the fidelity, for several years (from 1.44 to 12.6 years ago) to certain foraging grounds of the Macaronesia.

Introduction

Designing effective conservation plans to widely distributed species requires a robust understanding of the spatio-temporal distribution of the threats and the population units of interest (Webster *et al.,* 2002, Martin *et al.*, 2007, Wallace *et al.,* 2010). Sea turtles are a remarkable example of migratory species, for which understanding the complex relationships among various nesting sites, developmental and foraging areas is crucial to design effective conservation plans (Aguirre *et al*., 1998; Work *et a*l., 2003; 2004). Integration of multiple tools and techniques, including site-based monitoring, mark-

recapture studies, telemetry, genetic analyses and stable isotope analyses, can facilitate definitions of population segments at multiple biological and spatial scales to address different management and research demands (Wallace *et al*., 2010).

Distributed circumglobally in subtropical and warm temperate regions, the loggerhead sea turtle (*Caretta caretta*) is a long-lived, slow to mature marine megavertebrate, characterized by long migrations that span entire oceans with several ontogenetic shifts (Bolten, 2003; Bowen, 2005). The species is currently categorized as "Vulnerable" by the International Union for Conservation of Nature (IUCN, 2017) and included in the European Habitats Directive (92/43/CEE), as well as other national and regional conservation regulations (such as the *Listado de Especies Silvestres en Régimen de Protección Especia*l; *Catálogo Español de Especies Amenazadas* - Royal Decree 139/2011 - and the Canary Catalogue of Protected Species - Decree 20/2014).

Following hatching in nesting beaches, turtles swim offshore and become entrained in ocean current systems that may carry them to distant foraging areas in the oceanic waters. During this oceanic phase, that lasts for more than a decade, western Atlantic loggerhead turtles inhabit the north Atlantic gyre (Bolten, 2003; Bjorndal *et al*., 2000; Bolten *et al*., 1998; Bowen *et al.,* 2005; Monzón-Argüello *et al.,* 2009). Once juveniles are near to reach sexual maturity, with sizes between 40-60 cm of Curve Carapace Length (CCL; Bjorndal *et al.,* 2000; 2001), they recruit to zones closer to their natal areas (Hopkins-Murphy *et al*., 2003; Bowen *et al*., 2005). These areas are typically located in coastal neritic waters, although some individuals can remain in the oceanic environment or shuttle between oceanic-neritic habitats (Hatase *et al*., 2002; Hawkes *et al*., 2006; Bowen *et al*., 2005; Hatase *et al*., 2007; McClellan *et al*. 2007; Cardona *et al*. 2017). The recruitment to neritic habitats is thought to be triggered by improved diving performance and by increasing food demands (Bolten, 2003) and is usually accompanied by a shift in diet from epipelagic to benthic preys (Bjorndal, 1997). Once sexual maturation is reached, between 22.5-39 years for females and 26-42 years for males (Godley *et al*., 2003; Avens *et al*., 2015), turtles switch from subadult to adult foraging habitats, undertaking breeding migrations to natal nesting beaches every 2-3 years (Hopkins-Murphy *et al*., 2003). During a single nesting season, each female lays several clutches of numerous eggs (ca. 100 eggs per clutch) on specific sandy beaches (Hamann *et al.,* 2003).

Due to the philopatric behavior of marine turtles, maternally transmitted mitochondrial DNA (mtDNA) has been successfully used for resolving questions about population structure, allowing the identification of 19 demographically independent Management Units (MU) in the Atlantic Ocean and the Mediterranean Sea (Figure 1; Shamblin *et al*., 2014). Connectivity among nesting populations and foraging grounds has been investigated using a method called *Mixed Stock Analysis* (MSA) that estimates the proportional contribution of each rookery to a mixed foraging ground, considering population size as *prior* information (Bolker *et al*., 2007).

Loggerhead sea turtle is the most common sea turtle species in Macaronesia, a biogeographical region located in the Eastern Atlantic that comprises five archipelagos: Azores, Madeira, Selvagen, Canaries and Cabo Verde (Figure 2; Marco *et al.,* 2011). Around the four first archipelagos, juveniles from different rookeries (source populations) are found in mixed stocks, with individuals originated in America and Africa (Bolten *et al*., 1998; Carreras *et al*. 2006; Monzón-Argüello *et al*. 2009). Juvenile loggerheads from the Canary Islands present a mean size of 36.00 cm (sd = 11.10) and predominantly inhabit the oceanic waters (Varo-Cruz *et al*., 2016). In this area, Orós *et al*. (2016) found that the main causes of admission of turtles in the Wildlife Rescue Center (WRC) of Gran Canaria between 1998 and 2014 were marine debris (50.81%) and fisheries (11.88%).

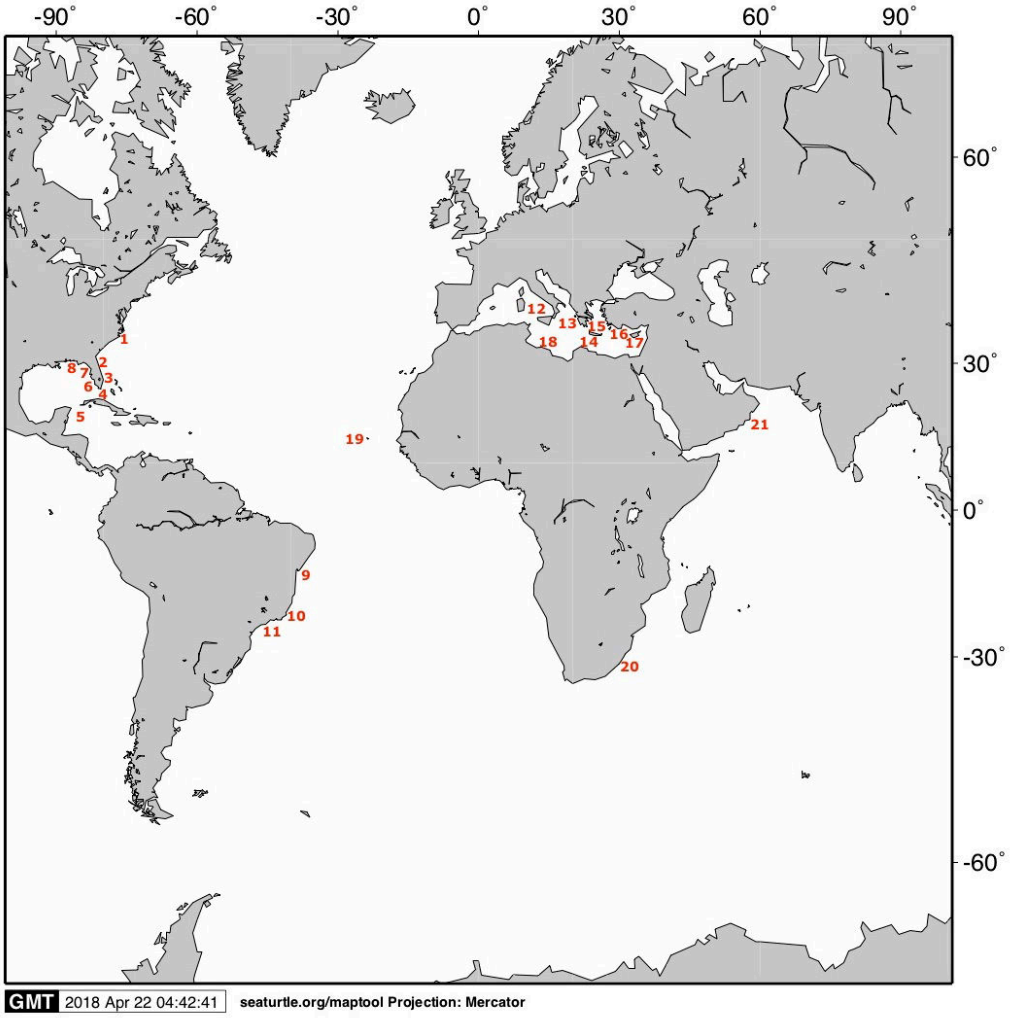


Figure 1: Rookeries identify by Shamblin et al. 2014: (1) Northern U.S.A; (2) Central Eastern Florida; (3) Southeastern Florida; (4) Cay Sal, Bahamas and Dry Tortugas, Florida; (5) Quintana Roo, Mexico and Cuba; (6) Keewdin Island, Florida (7) Casey Key, Florida (8) Northwestern Florida; (9) Sergipe and Bahía, Brazil; (10) Espiritu Santo, Brazil; (11) Rio de Janeiro, Brazil; (12) Calabria, Italy; (13) Western Greece; (14) Crete, Greece; (15) Dalyan and Dalaman, Turkey; (16) Western Turkey; and (17) Eastern Mediterranean rookeries (Israel, Lebanon and Cyprus); (18) Libya and Tunisia; (19) Cabo Verde; (20) Tongaland, KwaZulu-Natal, South Africa; (21) Masirah, Oman. Map created using Maptool (www.seaturtle.org/maptool).

Furthermore, the second largest nesting aggregation of loggerheads in the Atlantic Ocean is found at the Cabo Verde Islands, which is also the only major rookery in Western Africa (Monzón-Argüello *et al*., 2010). This rookery faces serious conservation threats that include substantial illegal harvest of eggs and adult females and harvest or incidental capture in fisheries (López-Jurado *et al*., 2000; Marco *et al*., 2011). Cabo Verde represents an independent MU (Monzón-Argüello *et al*., 2010; Shamblin *et al.,* 2014) and

juveniles from this rookery have been genetically identified at several feeding grounds of the Eastern Atlantic (Monzón-Argüello *et al.,* 2010). Nevertheless, 43.00% of Cabo Verdean juveniles are not found in the studied feeding areas and could be feeding at unknown foraging grounds, or alternatively, could have been eliminated from the metapopulation due to mortality in the early stages or in fisheries (Monzón-Argüello *et al*., 2010).

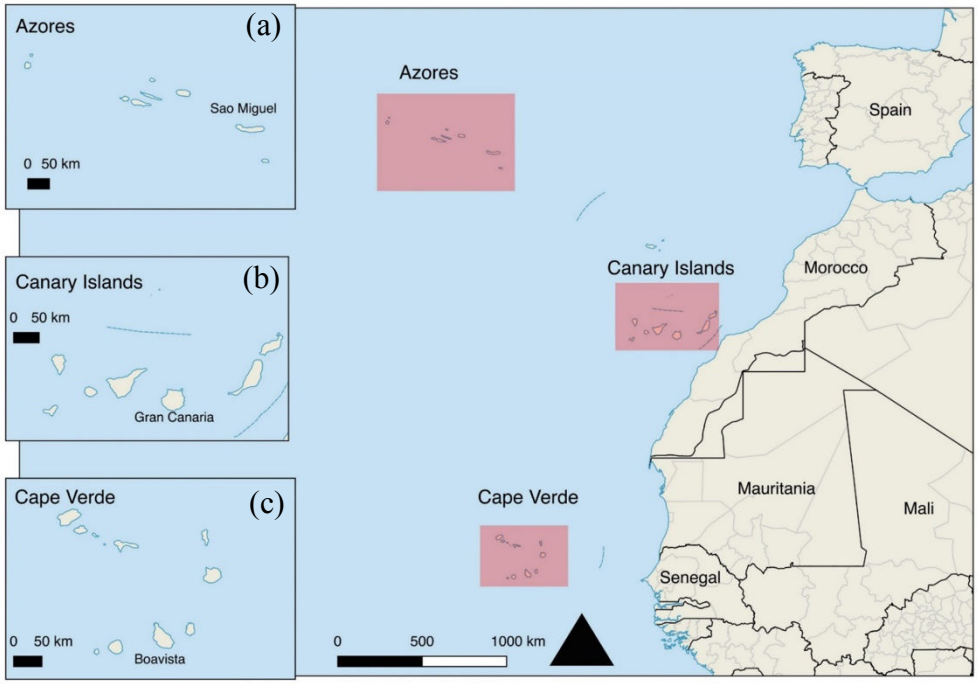


Figure 2: Map of the three studied Macaronesian archipelagos: (a) Azores; (b) Canary Islands and (c) Cabo Verde.

The life cycle of adult loggerhead turtles from Cabo Verde is characterized by two foraging strategies: females using neritic habitats of the north-western coast of Africa, of Guinea and Sierra Leone (12.50% of adult females) and oceanic females foraging in the upwelling area between the archipelago and mainland Africa (87,50% of adult females; Eder *et al.,* 2012; Varo-Cruz *et al*., 2013; Vieira *et al*., 2014). Hence, the vast majority of Cabo Verdean females are feeding in sub-optimal habitats, mostly restricted to the territorial waters of Mauritania and Senegal, but also waters of The Gambia, Guinea and Guinea Bissau (Hawkes *et al*., 2006).

The spatio-temporal connectivity among the Macaronesian foraging grounds for both juvenile and adult loggerheads is poorly known. Some juveniles that inhabit the Canarian

waters may have been feeding previously around northern Macaronesian archipelagos. Similarly, some Cabo Verdean turtles may feed around other Macaronesian archipelagos during the juvenile stage (eg. Azores and the Canary Islands; Monzón-Arguello *et al.,* 2010).

Here we analyzed a fragment of the mtDNA control region (Abreu *et al*., 2006) and stable isotopes ratios ($\delta^{13}C$ and $\delta^{15}N$) in order to determine the foraging habits and the spatio-temporal connectivity of loggerheads feeding in Macaronesian waters. We used mtDNA sequences to investigate the origin of juveniles feeding in Azores, Madeira and the Canary Islands. Foraging habits and movements were traced using stable isotope ratios (DeNiro & Epstein, 1978; 1981). Basically, the ratio $^{13}C/^{12}C$ ($\delta^{13}C$) is mainly used to identify feeding location (DeNiro & Epstein, 1978, 1981; Graham *et al*., 2010) due to differences in isotopic values between the primary producers of oceanic and neritic habitats, while the ratio $\delta^{15}N/^{14}N$ ($\delta^{15}N$) serves as indicators of consumer's trophic position but also varies regionally according to the N fixation/denitrification balance (Somes *et al.,* 2010).

Methods

Sample collection

Loggerheads of the Canary Islands

During 2015, we sampled 23 untagged loggerhead juveniles admitted in the WRC og Gran Canaria Island. For the genetic analysis, 2 ml of blood were collected from the dorsal cervical sinus (Owens & Ruiz, 1980) in heparin lithium and stored in 96% ethanol at -20 °C. Carapace scute samples for stable isotope analysis were collected from the central region of the third right lateral carapace scute using sterile 6 mm biopsy punches (Reich *et al.,* 2007; López-Castro *et al*., 2014). Scute samples were stored inside the biopsy punch in plastic bags at -20 °C. Stranding location, date of admission, admission cause and minimum curve carapace length (minCCL) were recorded for all the juvenile turtles (Supplementary material Table S1). After transforming CCL in Straight Carapace Length (SCL) using the equation estimated for juvenile loggerheads of the Canary Islands (Varo-Cruz *et al*., 2016), the age of each turtle was calculated using the smoothing spline model estimated in Avens *et al*. (2013).

Loggerheads from Cabo Verde

We sampled a total of 105 female loggerheads nesting in Boavista Island (Republic of Cabo Verde) during the nesting seasons (June to September) of 2015 and 2017 (Table 1).

The connectivity among Cabo Verdean foraging grounds and other Macaronesian juvenile foraging grounds was investigated sampling untagged nesting females, as these individuals have a higher probability to be new recruits (Table 1). To represent the complete size range, we sampled females from three different size classes (CCL ≤ 83 cm; 83 < CCL < 86 cm; CCL ≥ 86 cm; Table 1).

To investigate oceanic and neritic adult foraging areas in Cabo Verdean waters, recaptured females, tagged in Boavista seven years ago or more, were sampled (n = 44; Table 1). This recapture time was established based on the represented time (≈ 6.5 years) of diet chronological history in an adult carapace scute sample (see stable isotope analysis for further details; Vander Zanden *et al.,* 2013). As there is a foraging dichotomy linked to turtle size, with smaller turtles feeding in the open ocean and larger individuals feeding in coastal habitats, we sampled females ≥ 86 cm of CCL to obtain a similar proportion of turtles of both habitats (Eder *et al*., 2012).

Blood and scute samples were collected and conserved using the same procedure than in loggerheads from the Canary Islands. For all individuals, nesting date, nesting beach, CCL and the passive integrated transponder number (PIT; Avid, Norco, California, USA) were recorded. Untagged turtles were tagged with a PIT tag injected into the right front flipper.

Genetic analysis

Genomic DNA was isolated from samples (n = 128) using the E.Z.N.A.® Blood DNA Mini Kit (OMEGA bio-tek) following manufacturer's protocols. A 900-base pair (bp) fragment of the mtDNA control region was amplified by the polymerase chain reaction (PCR) in the totality of the samples using the primers LCM15382 (5'-GCTTAACCCTAAAGCATTGG- '3) and H950 (5' -GTCTCGGATTTAGGGGTTTG-3'; Abreu-Grobois *et al.,* 2006). Concentration and quality of extracted DNA was determined whit the Thermo Scientific NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) before undergo PCR reactions. PCR reactions were typically performed in 20 μL under the following conditions: an initial denaturation step at 94 °C for 2

minutes; followed by 40 cycles of 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for 1 minute; with a final extension at 72°C for 5 minutes. Generally, 1 µl of the DNA extraction (≈ 20 ng) was used in 19 µl of the total volume of the PCR mixture, which contained 0.4 µM of each primer, 0.25 mM dNTPs, 0.6 U of Taq DNA polymerase, 1 x PCR buffer and 2mM $MgCl_2$. For analyzing the result of the PCR reaction, we used an agarose gel electrophoresis (1.25%) with 1x TAE buffer, adding 2.5 µL of SYBR® Safe (Thermo Fisher Scientific), to allow the DNA to be visualized under UV light. 2 µL of loading dye were also added to the PCR samples for the electrophoresis. The voltage used was 90V for 40 minutes. Negative controls were included in each batch of PCR amplifications and sequencing reactions to detect contamination. PCR products were sent to a private laboratory (Secugen S.L., Spain) to be purified and sequenced in both forward and reverse directions. The chromatograms were aligned using the BioEdit Sequence Alignment Editor software version 7.2.6.1 (Copyright© 1997-2017, Hall 1999). The obtained sequences were classified according to standardized nomenclature (Archie Carr Center for Sea Turtle Research, ACCSTR; http://accstr.ufl.edu/files/cclongmtdna.pdf).

Table 1: Untagged and recaptured females sampled in Cabo Verde during 2015 and 2017. For untagged females, three different size classes were sampled. CCL: Curve Carapace Length.

Cabo Verde	2015	2017	Total
Group 1. Untagged			
CCL ≤ 83 cm	19	-	19
83 < CCL < 86 cm	14	6	20
CCL ≥ 86 cm	10	12	22
Group 2. Recaptured			
CCL ≥ 86 cm	8	36	44

Haplotype frequencies, haplotype diversity (h) and nucleotide diversity (π) of the studied groups were estimated using Arlequin v.3.5 (Excoffier *et al.,* 2005). Analyses used the Tamura–Nei model designed for control region sequences (Tamura & Nei, 1993) to estimate sequence divergence. Genetic differences between Atlantic and Mediterranean

nesting populations and foraging grounds (Bowen *et al.*, 1994; 2004; Carreras *et al*., 2007; Chaieb *et al*., 2010; Clusa *et al*., 2013; 2014; Garofalo *et al*., 2009; Monzón-Argüello *et al*., 2010; Reis *et al*., 2010; Saied *et al.,* 2012; Shamblin *et al.* 2011; 2012;; 2014; Yilmaz *et al.,* 2011, Table S3 and S4) were determined using F statistic and the exact test of populations differentiation with a statistical significance (0.05) obtained over 10,000 permutations using the program Arlequin v.3.5 (Excoffier *et al*., 2005). Similarly, we compared the new data obtained for loggerheads stranded in the Canary Islands and from Cabo Verde with published data using the exact test of populations differentiation with a statistical significance (0.05) obtained over 10,000 permutations using the program Arlequin v.3.5 (Excoffier *et al*., 2005). As information for foraging grounds using the 900 bp fragment (Abreu-Grobois *et al.*, 2006) is still unpublished, the analyses that included other foraging grounds besides the Canary Islands (this study) trimmed our control region sequence alignments to 391 bp. Results of population differentiation tests were used to define the groups for the many to many MSA.

To determine the origin and connectivity among nesting populations of juveniles feeding in Macaronesia, we conducted a Bayesian many-to-many MSA that estimates the proportional contribution of each rookery to a mixed foraging ground, considering population size as *prior* information (Bolker *et al.,* 2007). The analysis included 19 Atlantic and Mediterranean rookeries and 9 foraging grounds to simultaneously estimates the origins and destinations of individuals in a metapopulation, taking into account nesting population sizes (Bowen *et al.*, 1994; 2004; Carreras *et al*., 2007; Chaleb *et al*., 2010; Clusa *et al*., 2013; 2014; Garofalo *et al*., 2009; Monzón-Argüello *et al*., 2010; Reis *et al*., 2010; Revelles *et al*, 2007b; Saied *et al.,* 2012; Shamblin *et al.,* 2011; 2012; 2014; Yilmaz *et al.,* 2011). After using the Markov chain Monte Carlo (MCMC) method to obtain the posterior distribution of the parameters of interest, the Gelman–Rubin diagnostic test was used to confirm convergence of the chains to the posterior distribution, with values less than 1.2 (Gelman and Rubin, 1992).

Individuals with haplotypes that had not been previously observed in any of the rookeries (‘orphan’ haplotypes) were removed from the analysis by the program as these are non-informative; however, they may indicate undersampled rookery or rookeries.

Stable isotope analysis

The time scale on which dietary information is represented by stable isotopes ratios varies with tissue type and depends on metabolic turnover (Tieszen *et al.,* 1983). The carapace scutes of sea turtles are composed of several keratin layers, the most recent layer being more internal and the older being most external (Kobayashi, 2001). The isotopic signals of keratinous tissue do not change once it is produced (Ayliffe *et al*., 2004), and consequently the isotopic signal of each carapace layer is thought to reflect the composition of the diet consumed when it was formed (Reich *et al*., 2007). The scute record retains a chronological history of resource use and is estimated to represent a minimum of 0.8 years in juveniles to a maximum of 6.5 years in adults (Reich *et al*., 2008; Vander Zanden *et al.,* 2013). Hence, the analysis of stable isotopes of carapace scutes offers a record of the foraging habitats used by individual turtles during several years and a document of ontogenetic habitat shifts (Reis *et al.,* 2010; Cardona *et al.,* 2009, 2010, 2017).

Juvenile and Cabo Verdean adult carapace scute samples (n = 105) were embedded in an optimum cutting temperature (OCT) formulation of water-soluble glycols and resins, frozen to −20 °C and cut with a cryotome into 30 μm layers to obtain a chronological sequence of habitat and diet changes, assuming that each 50 μm thick layer was expected to integrate a turtle's diet over about 3 months (Vander-Zander *et al*., 2010). Due to differences in scute thickness, different numbers of layers (8–59) were obtained. For Canarian juveniles, a number of 2-5 layers, that represented the whole scute thickness (11-35 layers), were analysed. For adult females from Cabo Verde, we analysed the external and the innermost layers (2-59 layer's position).

Samples were processed at *Centres Científics i Tecnològics de la Universitat de Barcelona*. Sections were kept into a stove at 60 °C overnight, rinsed daily with a 2:1 chloroform:methanol solution for five days and dried again for 24 h at 60 °C, for removing any paraffin trace. An average 0.3 mg of sample was used for stable isotope analysis of carapace samples. Samples were weighed into tin cups, combusted at 1000 °C in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA, Thermo Finnigan, Bremen, Germany). Stable isotope abundances were expressed in δ notation according to the following expressions (Post, 2002):

$\delta^{13}C = ([R_{sample} / R_{standard}] - 1) \times 10^3$

$\delta^{15}N = ([([R_{sample} / R_{standard}] - 1) \times 10^3,$

where R_{sample} and $R_{standard}$ are the corresponding ratios of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standards used for ^{13}C and ^{15}N determination were Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (air), respectively.

In order to study the movements of the turtles around different habitats of Macaronesia during their life, isotopic values from Azores, Canary Islands and Cabo Verde were compared.

For Azores, we used a previous study with loggerheads (Pajuelo *et al*., 2010), transforming the epidermis isotopic values to carapace scute values using the epidermis to carapace discrimination factor estimated in Vander-Zander *et al.,* (2014).

According to the stable isotope ratios of several preys (*Sepia officinalis, Octopus vulgaris, Pelagia noctiluca, Sardina pilchardus*; Monzón-Argüello *et al*., 2018) and the discrimination factor for the loggerhead sea turtle (Reich *et al*., 2008), $\delta^{13}C‰$ scute values of −16.3 ± 1.2‰ (mean ± SD) were expected for turtles eating *Octopus vulgaris* and values of −18.2 ± 0.7‰ (mean ± SD) were expected for turtles eating *Sardina pilchardus.* Then, we classified each carapace layer of Canarian turtles in oceanic (values lower than -16.34 ± 1.24 $\delta^{13}C$ ‰, mean ± SD) or neritic (values higher than -16.34 ± 1.24 $\delta^{13}C$ ‰, mean ± SD).

Using the values estimated in Cardona *et al*. (2017), layers from the Cabo Verdean females were classified as oceanic (-17.98 ± 0.54 $\delta^{13}C‰$ and 10.28 ± 1.00 $\delta^{15}N‰$, mean ± SD;) or neritic (-15.99 ± 0.75 $\delta^{13}C‰$ and 12.50 ± 1.38 $\delta^{15}N‰$, mean ± SD).

All statistical analyses were implemented in R 3.4.3 language (R Core Team; 2017).

Results

Mean body size in curve carapace length of juveniles of the Canary Islands was 43.83 ± 14.54 cm (mean ± SD, range = 22.7–79.0 cm, n = 23, Figure S1 and Table S1).

Genetic analysis

We detected 7 haplotypes in the Canary Islands foraging group (n = 23; Table S1), all of them previously described. The CC-A1.1 haplotype was the most frequent (39.13% relative frequency), while other haplotypes and their frequencies were as follows: CC-A1.3 (26.09%), CC-A2.1 (17.39%), CC-A3.1 (4.35%), CC-A10.1 (4.35%), CC-A11.1 (4.35%) and CC-A17.1 (4.35%). Although these represent the first mtDNA long fragment sequences (900 bp) for loggerheads in the Canary Islands, the comparison of the trimmed sequences (391 bp) with those previously published (Monzón-Argüello *et al*., 2009; 2012) revealed no genetic differentiation (exact p = 0.21214±0.0130). For analyses considering the short mtDNA short fragment sequences, we therefore pooled all the short sequences available for the Canary Islands (n = 205).

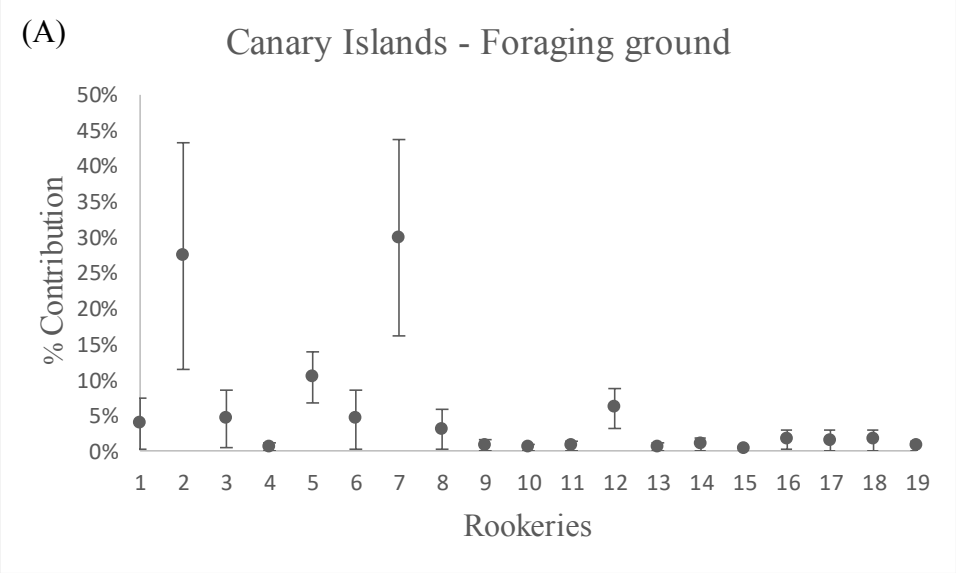
The genetic analysis of Cape Verdean females showed 9 haplotypes: CC-A1.3 (54.38%), CC-A17.1 (28.07%), CC-A1.4 (6.14%), CC-A1.7 (4.38%), CC-A2.1 (3.51%) CC-A11.6 (0.87%), CC-A1.1 (0.87%), CC-A11.2 (0.87%), CC-A17.2 (0.87%); all of them formerly described for the population (Shamblin *et al*., 2014). The comparison of these new data with those previously published revealed no genetic differences using the exact test of population differentiation (exact p = 0.07405±0.0106). We therefore pooled all Cape Verde sequences for subsequent analyses (n = 330).

As expected, juveniles of the Canary Islands showed a higher haplotype and nucleotide diversities (h = 0.673±0.030 and π = 0.026±0.013) than adult females from Cabo Verde (h = 0.449±0.024 and π = 0.0028±0.002), since they constitute a mixed stock with juveniles coming from different nesting populations.

Results of the F statistic and the exact test of population differentiation defined 19 Atlantic and Mediterranean rookeries for the posterior MSA (Table S4, S5, S6 and S7): (1) North USA; (2) Central East Florida; (3) South East Florida; (4) Dry Tortugas, Florida and Cay Sal, Bahamas; (5) Quintana Roo, Mexico and Cuba; (6) Keewdin Island, Florida; (7) Casey Key, Florida; (8) Northwestern Florida; (9) North Brazil; (10) Espiritu Santo, Brazil; (11) Rio de Janeiro, Brazil; (12) Cabo Verde; (13) Calabria, Italia; (14) Western Greece; (15) Crete; (16) Dalyan and Dalaman, Turkey; (17) Western Turkey; (18) Eastern Mediterranean (Israel, Lebanon and Cyprus); and (19) Libya and Tunisia.

Based on the genetic differentiation analyses, 9 foraging groups were considered for the MSA (Table S4, S5, S6 and S7): (1) Azores, (2) Madeira, (3) Canary Islands, (4) Western Mediterranean (Andalusia and Algerian Sea), (5) East Spain (Catalan coast and Balearic Islands), (6) Central Mediterranean (Ionian, South Adriatic and North Adriatic), (7) Tyrrhenian Sea, (8) Lampedusa and (9) South Levantine sea. Orphan haplotypes, non-detected previously in any rookery, were removed from the MSA by the program.

Foraging ground centric many-to-many MSA revealed that, in the Canary Islands foraging ground, juveniles come basically from Northwestern Atlantic (Florida) and a less than a 10.00% from Cabo Verde (Figure 3A). Similarly, in Azores and Madeira, juveniles come mainly from the Northwestern Atlantic and in a less measure from Cabo Verde (Figures 3B and 3C).



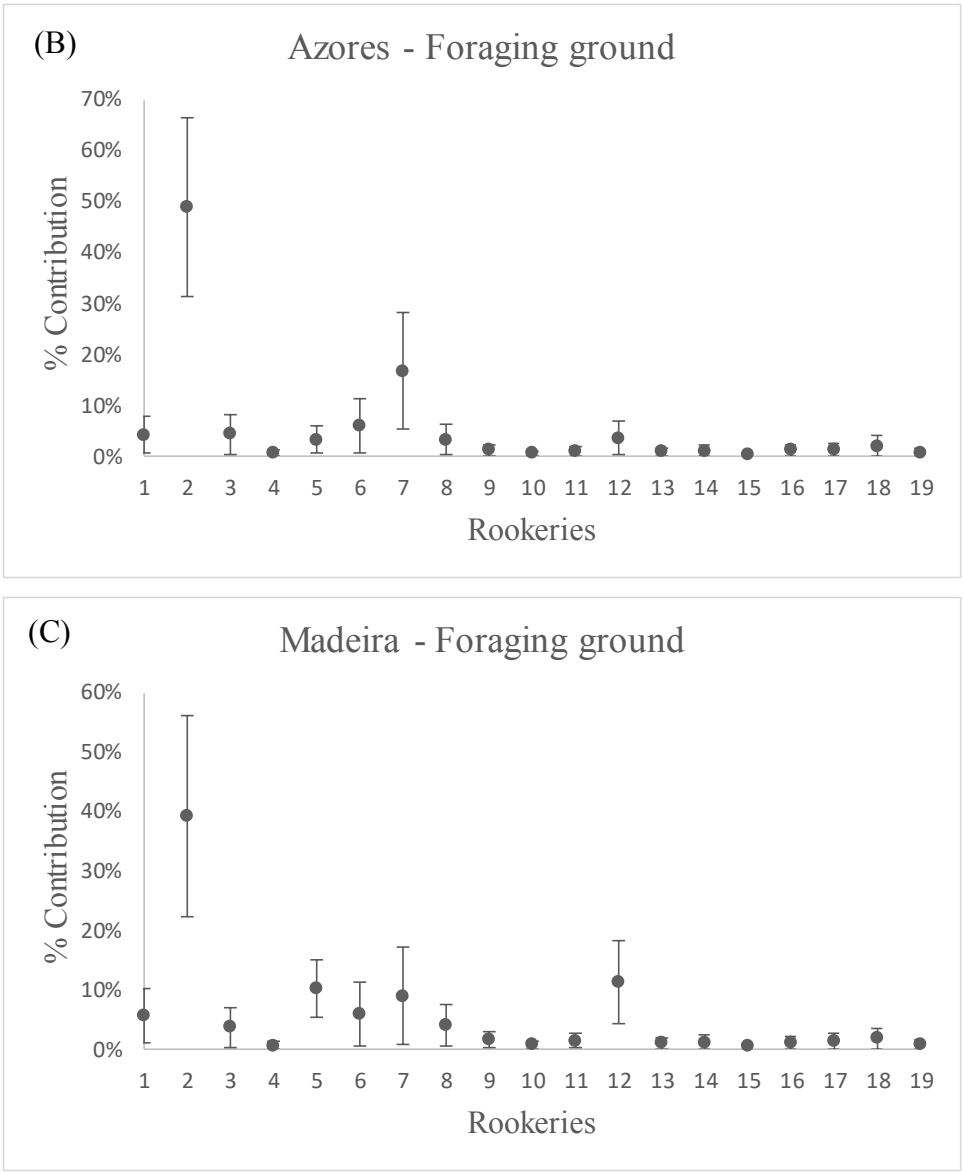


Figure 3: Foraging ground centric many-to-many MSA results (mean and SD) for Canary Islands (A), Azores (B) and Madeira (C) foraging grounds. Each number represents a rookery: (1) North USA; (2) Central East Florida; (3) South East Florida; (4) Dry Tortugas, Florida and Cay Sal, Bahamas; (5) Quintana Roo, Mexico and Cuba; (6) Keewdin Island, Florida; (7) Casey Key, Florida; (8) Northwestern Florida; (9) North Brazil; (10) Espiritu Santo, Brazil; (11) Rio de Janeiro, Brazil; (12) Cabo Verde; (13) Calabria, Italia; (14) Western Greece; (15) Crete, Greece; (16) Dalyan and Dalaman, Turkey; (17) Western Turkey; (18) Eastern Mediterranean; and (19) Libya and Tunisia.

Rookery centric many-to-many MSA showed that a proportion of juveniles from Cabo Verde rookery recruit mainly in Macaronesia (Azores, mean ± SD = 0.072 ± 0.077%; Madeira mean ± SD = 0.205 ± 0.143 % and Canary Islands mean ± SD = 0.099 ± 0.054 %) and in another unknown foraging grounds (Figure 4).

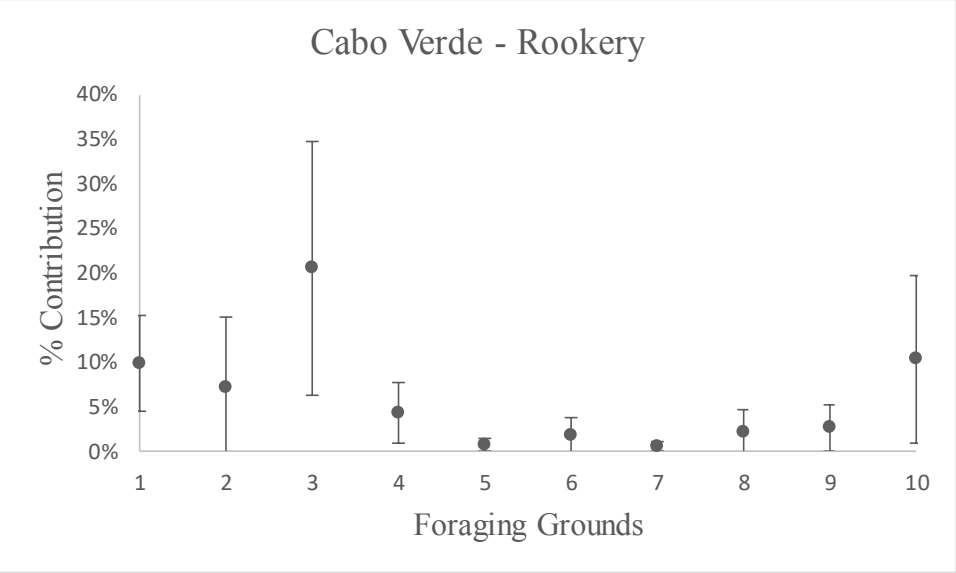


Figure 4: Results of rookery centric many-to-many MSA (mean and SD) for Cabo Verde rookery contribution to Atlantic and Mediterranean foraging grounds: (1) Canary Islands; (2) Azores; (3) Madeira; (4) Western Mediterranean; (5) East Spain; (6) Tyrrhenian Sea; (7) Central Mediterranean; (8) South Levantine; (9) Lampedusa; (10) Unknown.

Stable isotope analysis

Based on the $\delta^{13}C‰$ stable isotope values of the innermost layer (most recent layer), Cape Verdean females were classified as oceanic (79.05%) or neritic (20.95%; Table S8), defining the two isospaces for Cabo Verde. The isotopic space for Azores was -16.04 ± 1.10 $\delta^{13}C‰$ and 5.36 ± 0.61 $\delta^{15}N‰$ (mean ± SD; Figure 5). Using these results, four isospaces were identified based on the $\delta^{13}C‰$ and $\delta^{15}N‰$: (1) Azores; (2) Oceanic Canary Islands; (3) Oceanic Cabo Verde and (4) Neritic Cabo Verde (Figure 5). Neritic isospace from Canary Islands was not represented because only one juvenile was found with neritic values in the innermost carapace layer (Table S2).

In the Canary Islands, most turtles were oceanic (95.65%) and one individual with possible neritic values (4.34%), according to the $\delta^{13}C$ values of the innermost layer (Table S2; Figure S2).

The analysis of carapace scute layers of turtles of the Canary Islands revealed that 60.89% of the juveniles sampled presented only oceanic isotopic values of Canarian waters (Table S2). The remaining 39.12% presented values of three other foraging grounds, including the following: oceanic Canary Islands and neritic Canary Islands (4.34%), Azores and

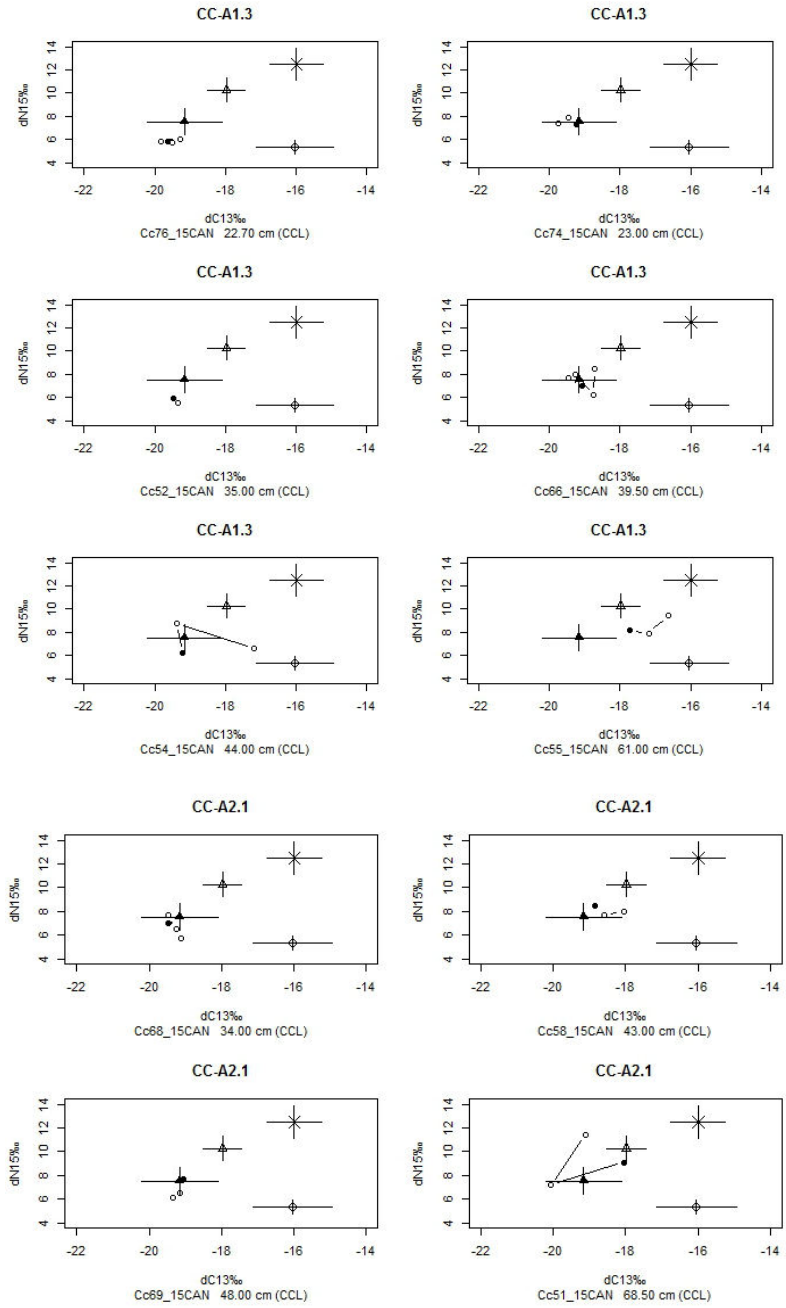
oceanic and Canary Islands (8.69%), oceanic Canary Islands and oceanic Cabo Verde (21.74%), oceanic Cabo Verde and neritic Canary Islands (4.34%).

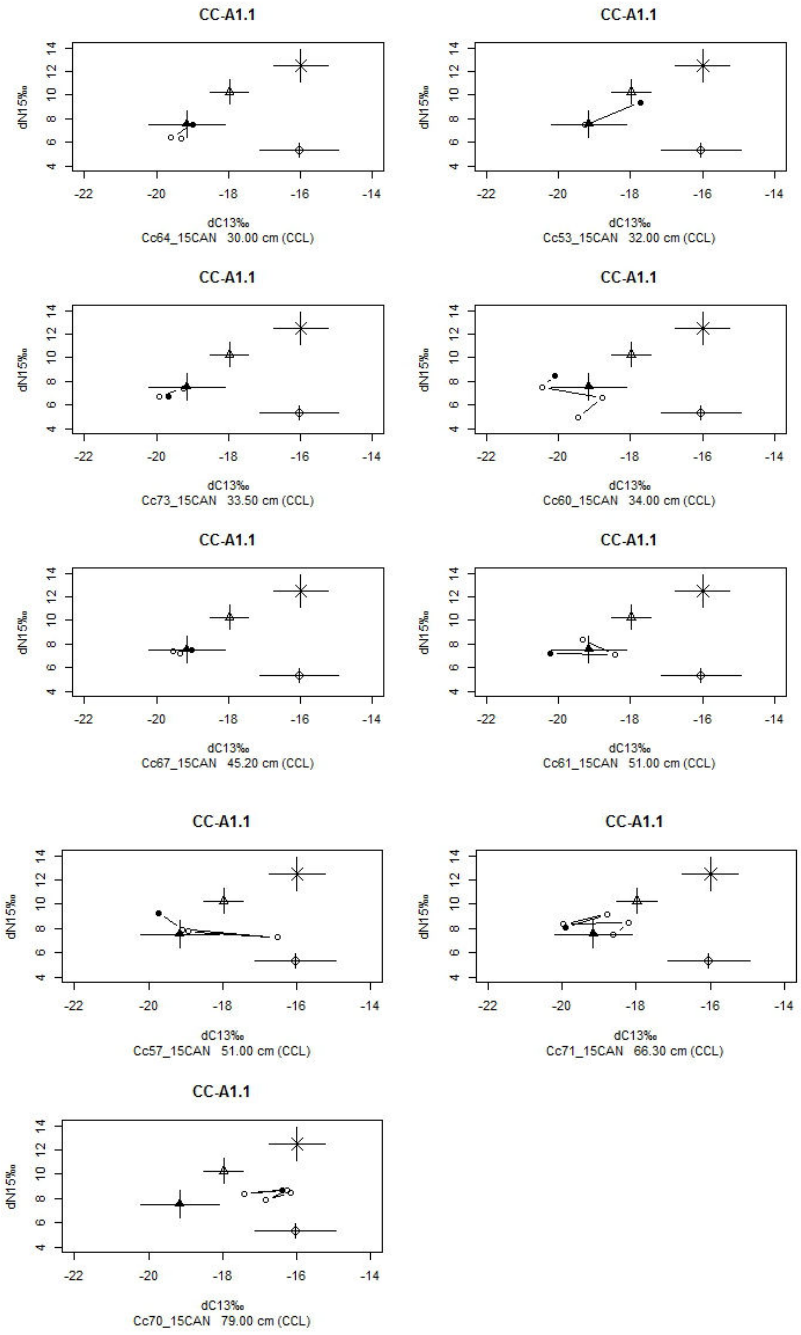
Cc55_15CAN showed 2 layers possible with neritic signals from Canary Islands in layers formed 5.76 and 9.36 years ago, and a recent layer with oceanic Canarian values.

8.69% of the sampled juveniles (2.44% of the layers) have at least one analyzed layer with stable isotope ratios consistent with foraging off Azores. Individual Cc54_15CAN (mtDNA haplotype CC-A1.3) presented an isotopic signal formed 6.12 years ago that corresponded to Azores together with other layers with oceanic values from the Canary Islands. Turtle Cc57_15CAN (CC-A1.1) presented an isotopic ratio value corresponding to Azores in a layer formed 3.24 years ago, and the rest of the analyzed layers with oceanic values from the Canary Islands (Figure 5; Table S2).

A 26.08% of the sampled juveniles (14.63% of the layers) have an isotopic value from Cabo Verde at least in one of their studied layers. Juvenile Cc53_15CAN (CC-A1.1) and Cc72_15CAN (CC-A17.1) had an oceanic isotopic signal from Cabo Verde in a 1.08 years old carapace layer. Turtle Cc59_15CAN (CC-A10.1) presented an oceanic isotopic signal from Cabo Verde formed 6.84 years ago. Turtle Cc51_15CAN (CC-A2.1) had 2 oceanic values from Cabo Verde in layers formed 1.08 and 5.4 years ago (Figures S4 and S5).

The other 4.34% of the sampled juveniles, that presented values from oceanic Cabo Verde and neritic Canary Islands, was formed by juvenile Cc70_5CAN (CC-A1.1), the largest juvenile sampled (79 cm minCCL). In this individual, three of the analyzed layers, formed 1.08, 6.84 and 12.6 years ago, respectively, had isotopic signals similar to neritic Canary Islands. Interestingly, the other 2 layers formed 3.96 and 9.72 years ago had oceanic signals possibly from Cabo Verde (Figure 5; Table S2).


CC-A1.3
dN15‰
dC13‰
Cc76_15CAN 22.70 cm (CCL)
CC-A1.3
dC13‰
Cc74_15CAN 23.00 cm (CCL)
CC-A1.3
dC13‰
Cc52_15CAN 35.00 cm (CCL)
CC-A1.3
dC13‰
Cc66_15CAN 39.50 cm (CCL)
CC-A1.3
dC13‰
Cc54_15CAN 44.00 cm (CCL)
CC-A1.3
dC13‰
Cc55_15CAN 61.00 cm (CCL)
CC-A2.1
dC13‰
Cc68_15CAN 34.00 cm (CCL)
CC-A2.1
dC13‰
Cc58_15CAN 43.00 cm (CCL)
CC-A2.1
dC13‰
Cc69_15CAN 48.00 cm (CCL)
CC-A2.1
dC13‰
Cc51_15CAN 68.50 cm (CCL)


CC-A1.1
dN15‰
dC13‰
Cc64_15CAN 30.00 cm (CCL)
CC-A1.1
dN15‰
dC13‰
Cc53_15CAN 32.00 cm (CCL)
CC-A1.1
dN15‰
dC13‰
Cc73_15CAN 33.50 cm (CCL)
CC-A1.1
dN15‰
dC13‰
Cc60_15CAN 34.00 cm (CCL)
CC-A1.1
dN15‰
dC13‰
Cc67_15CAN 45.20 cm (CCL)
CC-A1.1
dN15‰
dC13‰
Cc61_15CAN 51.00 cm (CCL)
CC-A1.1
dN15‰
dC13‰
Cc57_15CAN 51.00 cm (CCL)
CC-A1.1
dN15‰
dC13‰
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CC-A1.1
dN15‰
dC13‰
Cc70_15CAN 79.00 cm (CCL)

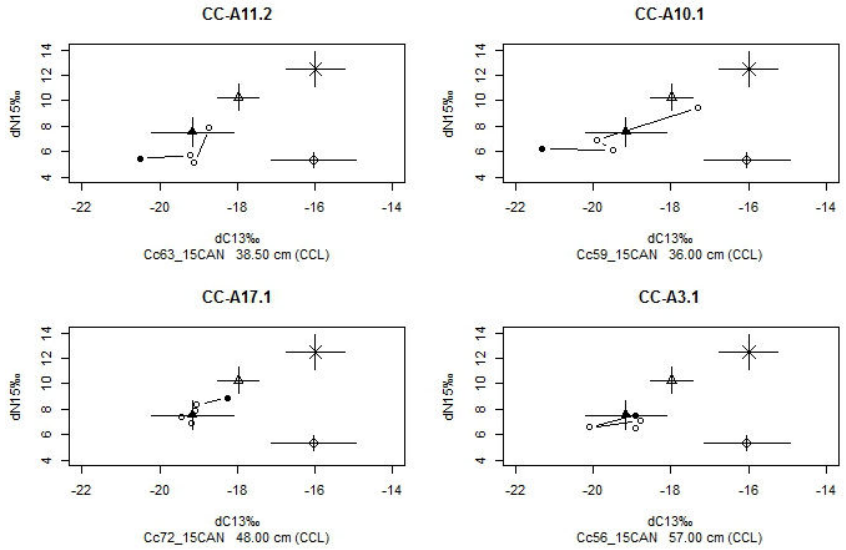

Figure 5: Values of isotopic ratios of carapace scute layers of juvenile loggerheads of the Canary Islands. The black point represents the innermost analysed layer (most recent layer). The four isotopic isospaces identified are included (mean and standard deviation): (1) neritic females from Cabo Verde (cross); (2) oceanic females from Cabo Verde (empty triangle); (3) Azores (empty point; Pajuelo *et al*., 2010) and (4) oceanic juveniles from the Canary Islands (black triangle).

Discussion

Loggerhead sea turtles are characterized by complex population structure that outcome from population overlap during migrations and sex-biased gene flow (Bowen *et al.*, 2005), resulting in a challenge for designing effective conservation measures (Ceriani *et al.,* 2012). Macaronesia, located in the Northeastern Atlantic, harbors several juvenile foraging grounds and the second major nesting aggregation for loggerheads in the Atlantic (Marco *et al*., 2011). Here, we combined, for the first time, two laboratory analyses - mtDNA sequences and stable isotopes- to explore the spatio-temporal connectivity among these areas.

Genetic analysis

F_{ST} results showed no genetic differences between foraging grounds of Andalusia and Algerian Sea; neither between Catalan Coast and Balearic Islands and among North Adriatic, South Adriatic and Ionian Sea. Despite Canary Islands, Azores and Madeira were not significantly different, these two foraging grounds were analyzed separately in the MSA in order to have a better resolution of the studied area (Table S6).

Juvenile loggerhead foraging grounds represent areas were turtles from different nesting beaches or rookeries live together (Bolker *et a*l., 2007). This was confirmed by the haplotype (h) and nucleotide diversities (π), that were always higher in the Canary Islands than in Cabo Verde.

Foraging ground-centric MSA revealed no contribution from Mediterranean nesting rookeries. The Mediterranean dominant currents and the strait of Gibraltar may act as a barrier, preventing that Mediterranean juveniles arrive to the Atlantic as have been previously suggested (Carreras *et al*., 2006, Revelles *et al*., 2007a, Monzón-Arguello *et al*., 2009). Loggerheads from the Canary Islands mainly came from Northwestern Atlantic (85%; Figure 3A), with a migration facilitated probably by the Gulf stream (Monzón-Arguello *et al*., 2009). Because loggerheads in the Canary Islands are mainly originated in the Eastern USA, a decline in nest abundance on origin beaches could have a negative impact on the number of juveniles around the Canary Islands, and *vice versa*.

Monzón-Argüello *et al*. (2012) suggested that storm driven surface currents may move juvenile turtles to locations outside their expected distribution. This could prevent juveniles from Cabo Verde to arrive to Western Atlantic, diverting them to Canary Islands, before the expected time (Monzón-Argüello *et a*l., 2012), using a different migratory route than American juveniles to reach this area. Interestingly, a low percentage of Cabo Verdean juveniles forage in Macaronesian waters (less than a 40%; Figure 4). Moreover, the rookery-centric MSA revealed that a huge number of individuals from Cabo Verde go to unknown areas during their pelagic stage (more than a 10%; Figure 4).

The genetic differentiation observed among the nesting populations with large mtDNA sequences allow a more precise definition of management units, which otherwise might have gone undetected (Shamblin *et al*., 2014). Although we analyzed our Canarian samples with the large mtDNA sequences, no information is available for other foraging grounds, limiting us to conduct the MSA with this larger fragment. Hence, we recommend analyzing Azores and Madeira juveniles with the larger sequences, as this might increase the resolution of turtle's origin and movements and conservation requirements.

Stable isotope analysis

Based on the $\delta^{13}C$ and $\delta^{15}N$ values of the innermost carapace layer, we were able to differentiate five foraging grounds: (1) Azores, (2) oceanic Canary Islands, (3) neritic

Canary Islands, (4) oceanic Cabo Verde and (5) neritic Cabo Verde. The neritic foragers from Cabo Verde actually forage on the African shelf (Eder *et al*., 2012). The isospace of neritic Canary Islands was not represented because was only found one individual with neritic values in the innermost layer (Cc70_15CAN). The isotopic differentiation within Macaronesian regions gives the opportunity to investigate movements among Azores, the Canary Islands and Cabo Verde.

The vast majority of the analyzed layers from juveniles stranded in the Canary Islands presented isotopic values that belong to the oceanic Canary Islands isospace (82.92%), with a small proportion having a signal from Cabo Verde (14.63%) and from Azores (2.44%). Similarly, the vast majority of the turtles stranded in the Canary Islands showed isotopic signal from the oceanic Canary Island isospace in all the studied layers (60.89%), with only a 18.19% having signals from two different isospace (Canary Islands-Cabo Verde, 39.11%; Canary Islands-Azores, 8.69%; oceanic Canary Islands-neritic Canary Islands, 4.34% Table S2). The unique turtle found in the Canary Islands with a neritic isotopic signal from the Canary Islands ($\delta^{13}C$ = -16.18‰ and $\delta^{15}N$ = 8.49‰) was the individual Cc70_15CAN that was also the largest in size (79.00 cm minCCL). The mtDNA sequence of this individual revealed a haplotype from the Northwestern Atlantic (CC-A1.1), then, the isotopic signal could indicate a permanent change in the habitat before coming back to areas near to its rookery (Table S2). The turtle Cc55_15CAN also could have neritic values from Canary Islands in older layers, having undertaken the opposite expected shift (neritic to oceanic habitat). This reversal process has already been documented (Cardona *et al*., 2017 and demonstrates that settlement is not a one-way process.

The isotopic signal from Azores appeared always in the oldest layers (Cc57_15CAN and Cc54_15CAN), suggesting that some turtles may have been foraging in Azores before arriving to the Canary Islands. Nevertheless, the low percentage of turtles with a signal from Azores in the oldest layers suggest that this strategy may not be common and that the transoceanic migration to the Eastern Atlantic may be quite direct until they reach the Canary Islands. Furthermore, the absence of isotopic values from Azores in the recent layers supports the telemetry results from previous studies, where no movements between the Canary Islands and Azores were detected (Varo-Cruz *et al*., 2016).

Oppositely, isotopic signals from Cabo Verde usually appeared in the most recent layers

(Cc51_15CAN, Cc53_15CAN, Cc70_15CAN and Cc72_15CAN), indicating that some individuals might move between Canary Islands and Cabo Verdean waters, as they stranded in the Canary Islands. Moreover, in all these individuals except Cc70_15CAN, the older layers above those with the signals from Cabo Verde had an oceanic signal from the Canary Islands. This suggest that turtles were foraging off the Canary Islands before moving to Cabo Verde (Table S2). As the vast majority of the turtles (81.81% of individuals) showed only stable isotope ratios consistent with foraging off the Canary Island, we could not analyze differences in the foraging areas used among different mtDNA haplotypes.

Besides not having samples to determine the isotopic values from Madeira, previous telemetry studies showed that Canarian loggerhead juveniles seldom approach Madeira (Varo-Cruz *et al*., 2016) and, because of that, isotopic values could neither be reflected in their carapace. This could be due to the opportunistic foraging behavior of loggerheads, feeding while traveling, and presumably using front-associated foraging opportunities as they encounter them, moving to areas with a higher primary production (Scales *et al*., 2015).

Our results highlight the transatlantic connectivity of turtles inhabiting the Macaronesian waters and show, for the first time, the fidelity, for several years (from 1.44 to 12.6 years ago; Table S2; Figure S4 and S5), to certain foraging grounds of the Macaronesia, where they may stay feeding before coming back near to their rookeries. The isotopic values showed that, in general, individuals only showed signals from one Macaronesian foraging ground, showing a fidelity to certain areas within the region where they may stay feeding before coming back near their nesting beaches.

In the last decade, stable isotopes ratios have been increasingly used as intrinsic markers to trace foraging habits and movements of wildlife populations (Ceriani *et al*., 2012). Although their use is a powerful and alternative technique to satellite telemetry to infer foraging grounds, interpreting the results is not always straightforward, because the method is only reliable when large differences exist among areas (Roscales *et al.,* 2011; Ceriani *et al*., 2012). Nevertheless, Ceriani *et al.* (2012) emphasized the need to validate the use of isotopic signatures with satellite telemetry in a subsample of individuals because oceanographic processes that can affect baseline stable isotopes ratios differ among ocean basins and geographical regions and, because of that, data interpretations

without validations could be misleading. Then, future studies should combine the analyses of isotopic signals with telemetry results to validate the results obtained in this study. In this study neritic values from Canary Islands could be confused by oceanic values from Azores, because of the lack of turtles found with the innermost layer with neritic values in Canary Islands, and also, because of the proximity of the values of both isospaces.

Conservation suggestions

In order to effectively conserve the complex network of rookeries and foraging grounds, it is necessary to know the connectivity among them and to assess the current levels of anthropogenic threats, evaluate conservation measures currently in place and design and implement efficient measures for the whole area of the population we want to preserve.

As the main cause of admission in WRC in the Canary Islands is the entanglement in marine debris (Orós *et al*., 2016), legal actions including the compulsory use of biodegradable raffia must be implemented in order to minimize the entanglement of sea turtles. Although the interactions with fisheries are not very common in this geographic area (Orós *et al*., 2016), potential interactions between juveniles that inhabit the canarian waters and fisheries gears can take place on waters outside the Canary Islands (eg. Cabo Verdean waters), as our results showed. Thus, due to the high migratory behavior of loggerhead sea turtle, it is vital that the Atlantic nations with habitats used by loggerhead turtles are encouraged as much as possible to ratify and implement international marine conservation plans to reduce as much as possible its anthropogenic pressures.

Conclusions

1. Loggerheads foraging in Canary Islands mainly come from US Western Atlantic.
2. Mediterranean nesting grounds do not contribute to Macaronesian foraging grounds.
3. Juvenile loggerhead turtles exhibit long term (several years) fidelity to the foraging grounds off the Canary Islands.
4. Some juvenile loggerhead turtles move frequently between the Canary Islands and the Cabo Verde archipelago.

5. Azores, Madeira and Canary Islands support distinct aggregations of foraging juveniles with little exchange.
6. For the first time it is demonstrated the fidelity of turtles to certain foraging grounds of the Macaronesia for several years (from 1.44 to 12.6), where they may stay feeding before coming back near to their rookeries.
7. The interpretation of stable isotope ratios can be obscured because of the existence of distinct areas with similar isotopic values, for example the oceanic foraging grounds off Azores with the neritic foraging grounds off the Canary Islands.
8. Turtles from the Canary Islands presenting neritic values are always bigger than 60 cm CCL, suggesting that older turtles shift from oceanic to neritic habitats.
9. As a transatlantic connectivity of turtles inhabiting the Macaronesia waters is showed, effective conservation measures need to be implemented to protect the complex network of rookeries and foraging grounds for this endangered species.

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Supplement material

Table S1: Genetic and stable isotope results from loggerhead juveniles (n=23) stranded in the Canary Islands. $\delta^{15}N$ and $\delta^{13}C$ values of the different carapace layers studied, from the innermost layer (Inner layer) to the outermost layer (Ext. layer) are shown. Stage means the habitat of the turtle: oceanic (O), neritic (N) and the changes it has undertook oceanic to neritic and back to oceanic (ONO); neritic to oceanic and neritic again (NON); and neritic to oceanic (NO). Abbreviations: Minimun curve carapace lenght (minCCL).

Turtle	**minCCL** (cm)	**Haplotype**	**δ15N‰** (1) Inner layer	**δ15N‰** (2)	**δ15N‰** (3)	**δ15N‰** (4)	**δ15N‰** (5) Ext. layer	**δ13C‰** (1) Inner layer	**δ13C‰** (2)	**δ13C‰** (3)	**δ13C‰** (4)	**δ13C‰** (5) Ext. layer	**Stage**
Cc76_15CAN	22.7	CC-A1.3	5.8	5.7	5.8	6.1		-19.6	-19.5	-19.8	-19.3		O
Cc74_15CAN	23.0	CC-A1.3	7.3	7.9	7.4			-19.3	-19.5	-19.8			O
Cc64_15CAN	30.0	CC-A1.1	7.5	6.4	6.3			-19.0	-19.6	-19.3			O
Cc53_15CAN	32.0	CC-A1.1	7.5	9.4				-19.3	-17.7				O
Cc73_15CAN	33.5	CC-A1.1	7.4	6.7	6.7			-19.3	-19.9	-19.7			O
Cc60_15CAN	34.0	CC-A1.1	5.0	6.6	7.5	8.5		-19.4	-18.8	-20.5	-20.1		O
Cc68_15CAN	34.0	CC-A2.1	7.0	7.7	6.5	5.7		-19.5	-19.5	-19.3	-19.1		O
Cc52_15CAN	35.0	CC-A1.3	6.0	5.5				-19.5	-19.4				O
Cc59_15CAN	36.0	CC-A10.1	6.2	6.2	6.9	9.5		-21.3	-19.5	-19.9	-17.3		O
Cc63_15CAN	38.5	CC-A11.2	5.5	5.7	5.1	7.9		-20.5	-19.2	-19.1	-18.7		O
Cc66_15CAN	39.5	CC-A1.3	7.0	7.7	8.0	6.3	8.4	-19.1	-19.5	-19.3	-18.7	-18.7	O
Cc58_15CAN	43.0	CC-A2.1	8.0	7.7	8.5			-18.1	-18.6	-18.9			O
Cc54_15CAN	44.0	CC-A1.3	6.6	8.8	6.2			-17.2	-19.4	-19.2			O
Cc67_15CAN	45.2	CC-A1.1	7.5	7.4	7.2			-19.0	-19.5	-19.3			O
Cc72_15CAN	48.0	CC-A17.1	8.9	8.3	7.9	7.0	7.4	-18.2	-19.0	-19.1	-19.2	-19.4	O
Cc69_15CAN	48.0	CC-A2.1	7.7	6.5	6.2			-19.0	-19.2	-19.3			O

Cc61_15CAN	51.0	CC-A1.1	8.4	7.2	7.2			-19.3	-18.4	-20.2			O
Cc57_15CAN	51.0	CC-A1.1	7.8	7.3	7.9	9.3		-18.9	-16.5	-19.1	-19.7		ONO
Cc56_15CAN	57.0	CC-A3.1	6.5	7.1	6.6	7.6		-18.9	-19.8	-20.1	-18.9		O
Cc55_15CAN	61.0	CC-A1.3	9.4	7.9	8.2			-17.7	-17.2	-16.6			NO
Cc51_15CAN	63.6	CC-A2.1	11.4	7.2	9.1			-19.1	-20.1	-18.1			O
Cc71_15CAN	66.3	CC-A1.1	8.1	9.2	8.4	8.5	7.5	-19.9	-18.8	-20.0	-18.2	-18.6	O
Cc70_15CAN	79.0	CC-A1.1	8.7	8.4	8.7	7.9	8.5	-16.4	-17.4	-16.3	-16.8	-16.2	NON

Table S2: Genetic and stable isotope results from loggerhead juveniles (n=23) stranded in the Canary Islands. $\delta^{15}N$ and $\delta^{13}C$ values of the different carapace layers. The age was calculated from the minCCL. Base distance is the distance between the analysed carapace layer and the base of the carapace, nearest layers are the most recent. Years ago, means the "age" of every studied layer and was calculated from the base distance. Stage means the habitat of the turtle in each layer: oceanic (O), neritic (N). Location was determinated from the isotopic values plots (Figure 5 in Results) and indicate the place where the carapace layer was formed Abbreviations: Minimun curve carapace lenght (minCCL).

Turtle	minCCL (cm)	Age (years)	Haplotype	δ15N‰	δ13C‰	Nº of layers	Layers	Base distance (µm)	Years ago	Stage	Location
Cc76_15CAN	22.70	0-2.5	CC-A1.3	6.08	-19.29	15	3+4	120	1.44	O	Can
Cc76_15CAN	22.70	0-2.5	CC-A1.3	5.83	-19.83	15	9+8	270	3.24	O	Can
Cc76_15CAN	22.70	0-2.5	CC-A1.3	5.70	-19.51	15	10	300	3.6	O	Can
Cc76_15CAN	22.70	0-2.5	CC-A1.3	5.80	-19.62	15	13+14+15	450	5.4	O	Can
Cc74_15CAN	23.00	0-2.5	CC-A1.3	7.44	-19.76	17	3	90	1.08	O	Can
Cc74_15CAN	23.00	0-2.5	CC-A1.3	7.86	-19.46	17	6+7	210	2.52	O	Can
Cc74_15CAN	23.00	0-2.5	CC-A1.3	7.27	-19.25	17	16+17	510	6.12	O	Can
Cc64_15CAN	30.00	0-2.5	CC-A1.1	6.29	-19.30	12	3	90	1.08	O	Can
Cc64_15CAN	30.00	0-2.5	CC-A1.1	6.42	-19.59	12	8	240	2.88	O	Can
Cc64_15CAN	30.00	0-2.5	CC-A1.1	7.49	-19.00	12	10	300	3.6	O	Can
Cc53_15CAN	32.00	0-2.5	CC-A1.1	9.39	-17.72	13	3	90	1.08	O	CV
Cc53_15CAN	32.00	0-2.5	CC-A1.1	7.49	-19.26	13	7	210	2.52	O	Can
Cc73_15CAN	33.50	0-2.5	CC-A1.1	6.74	-19.67	20	3	90	1.08	O	Can
Cc73_15CAN	33.50	0-2.5	CC-A1.1	6.74	-19.94	20	8+9	330	3.96	O	Can
Cc73_15CAN	33.50	0-2.5	CC-A1.1	7.37	-19.27	20	11	510	6.12	O	Can
Cc68_15CAN	34.00	2.5-5	CC-A2.1	5.74	-19.12	14	3	90	1.08	O	Can
Cc68_15CAN	34.00	2.5-5	CC-A2.1	6.52	-19.26	14	7	210	2.52	O	Can
Cc68_15CAN	34.00	2.5-5	CC-A2.1	7.67	-19.49	14	11	330	3.96	O	Can
Cc68_15CAN	34.00	2.5-5	CC-A2.1	7.00	-19.47	14	14+15	450	5.4	O	Can

Cc60_15CAN	34.00	2.5-5	CC-A1.1	8.50	-20.09	18	3	90	1.08	O	Can
Cc60_15CAN	34.00	2.5-5	CC-A1.1	7.54	-20.45	18	8	240	2.88	O	Can
Cc60_15CAN	34.00	2.5-5	CC-A1.1	6.58	-18.78	18	10	300	3.6	O	Can
Cc60_15CAN	34.00	2.5-5	CC-A1.1	4.95	-19.44	18	16+17	510	6.12	O	Can
Cc52_15CAN	35.00	2.5-5	CC-A1.3	5.96	-19.48	26	3	90	1.08	O	Can
Cc52_15CAN	35.00	2.5-5	CC-A1.3	5.51	-19.35	26	10+12	360	4.32	O	Can
Cc59_15CAN	36.00	2.5-5	CC-A10.1	9.45	-17.29	22	3	90	1.08	O	Can
Cc59_15CAN	36.00	2.5-5	CC-A10.1	6.91	-19.91	22	9	270	3.24	O	Can
Cc59_15CAN	36.00	2.5-5	CC-A10.1	6.17	-19.50	22	15	450	5.4	O	Can
Cc59_15CAN	36.00	2.5-5	CC-A10.1	6.19	-21.31	22	19	570	6.84	O	CV
Cc63_15CAN	38.50	2.5-5	CC-A11.2	7.87	-18.73	12	3	90	1.08	O	Can
Cc63_15CAN	38.50	2.5-5	CC-A11.2	5.11	-19.12	12	8	240	2.88	O	Can
Cc63_15CAN	38.50	2.5-5	CC-A11.2	5.69	-19.21	12	10	300	3.6	O	Can
Cc63_15CAN	38.50	2.5-5	CC-A11.2	5.48	-20.52	12	12	360	4.32	O	Can
Cc66_15CAN	39.50	5-7.5	CC-A1.3	8.44	-18.73	17	3+4	120	1.44	O	Can
Cc66_15CAN	39.50	5-7.5	CC-A1.3	6.26	-18.74	17	8	240	2.88	O	Can
Cc66_15CAN	39.50	5-7.5	CC-A1.3	8.02	-19.28	17	11	330	3.96	O	Can
Cc66_15CAN	39.50	5-7.5	CC-A1.3	7.68	-19.45	17	12	360	4.32	O	Can
Cc66_15CAN	39.50	5-7.5	CC-A1.3	6.99	-19.09	17	16	480	5.76	O	Can
Cc58_15CAN	43.00	5-7.5	CC-A2.1	8.51	-18.85	16	3	90	1.08	O	Can
Cc58_15CAN	43.00	5-7.5	CC-A2.1	7.69	-18.59	16	7+9	270	3.24	O	Can
Cc58_15CAN	43.00	5-7.5	CC-A2.1	7.99	-18.06	16	14+15	450	5.4	O	Can
Cc54_15CAN	44.00	5-7.5	CC-A1.3	6.19	-19.23	22	4	120	1.44	O	Can
Cc54_15CAN	44.00	5-7.5	CC-A1.3	8.76	-19.38	22	10	300	3.6	O	Can
Cc54_15CAN	44.00	5-7.5	CC-A1.3	6.57	-17.20	22	16+17	510	6.12	O	Az
Cc67_15CAN	45.20	5-7.5	CC-A1.1	7.20	-19.33	11	3	90	1.08	O	Can
Cc67_15CAN	45.20	5-7.5	CC-A1.1	7.43	-19.54	11	7	210	2.52	O	Can
Cc67_15CAN	45.20	5-7.5	CC-A1.1	7.53	-19.03	11	10+11	330	3.96	O	Can

Cc69_15CAN	48.00	5-7.5	CC-A2.1	6.17	-19.34	12	3	90	1.08	O	Can
Cc69_15CAN	48.00	5-7.5	CC-A2.1	6.48	-19.16	12	8	240	2.88	O	Can
Cc69_15CAN	48.00	5-7.5	CC-A2.1	7.66	-19.04	12	12	360	4.32	O	Can
Cc72_15CAN	48.00	5-7.5	CC-A17.1	7.41	-19.43	25	3	90	1.08	O	CV
Cc72_15CAN	48.00	5-7.5	CC-A17.1	6.95	-19.19	25	10	300	3.6	O	Can
Cc72_15CAN	48.00	5-7.5	CC-A17.1	7.93	-19.09	25	17	510	6.12	O	Can
Cc72_15CAN	48.00	5-7.5	CC-A17.1	8.33	-19.04	25	22	660	7.92	O	Can
Cc72_15CAN	48.00	5-7.5	CC-A17.1	8.86	-18.25	25	24+25	750	9	O	Can
Cc61_15CAN	51.00	7.5-10	CC-A1.1	7.16	-20.25	13	4	120	1.44	O	Can
Cc61_15CAN	51.00	7.5-10	CC-A1.1	7.15	-18.44	13	9	270	3.24	O	Can
Cc61_15CAN	51.00	7.5-10	CC-A1.1	8.36	-19.34	13	13	390	4.68	O	Can
Cc57_15CAN	51.00	7.5-10	CC-A1.1	9.30	-19.74	22	3+4	120	1.44	O	Can
Cc57_15CAN	51.00	7.5-10	CC-A1.1	7.93	-19.07	22	9	270	3.24	O	Can
Cc57_15CAN	51.00	7.5-10	CC-A1.1	7.29	-16.53	22	15	450	5.4	O	Az
Cc57_15CAN	51.00	7.5-10	CC-A1.1	7.76	-18.92	22	20	600	7.2	O	Can
Cc56_15CAN	57.00	12.5-15	CC-A3.1	7.55	-18.90	22	3+4	120	1.44	O	Can
Cc56_15CAN	57.00	12.5-15	CC-A3.1	6.58	-20.10	22	8	240	2.88	O	Can
Cc56_15CAN	57.00	12.5-15	CC-A3.1	7.14	-18.80	22	14	420	5.04	O	Can
Cc56_15CAN	57.00	12.5-15	CC-A3.1	6.53	-18.92	22	18+19	570	6.84	O	Can
Cc55_15CAN	61.00	12.5-15	CC-A1.3	8.16	-17.73	26	3+4	120	1.44	O	Can
Cc55_15CAN	61.00	12.5-15	CC-A1.3	7.93	-17.19	26	16	480	5.76	N	Can
Cc55_15CAN	61.00	12.5-15	CC-A1.3	9.42	-16.64	26	26	780	9.36	N	Can
Cc71_15CAN	66.30	12.5-15	CC-A1.1	7.54	-18.61	23	3	90	1.08	O	Can
Cc71_15CAN	66.30	12.5-15	CC-A1.1	8.45	-18.21	23	10	300	3.6	O	Can
Cc71_15CAN	66.30	12.5-15	CC-A1.1	8.39	-19.96	23	17	510	6.12	O	Can
Cc71_15CAN	66.30	12.5-15	CC-A1.1	9.18	-18.77	23	21	630	7.56	O	Can
Cc71_15CAN	66.30	12.5-15	CC-A1.1	8.08	-19.92	23	23	690	8.28	O	Can
Cc51_15CAN	68.50	15-17.5	CC-A2.1	9.10	-18.05	21	3	90	1.08	O	CV

Cc51_15CAN	68.50	15-17.5	CC-A2.1	7.22	-20.07	21	15	450	5.4	O	CV
Cc51_15CAN	68.50	15-17.5	CC-A2.1	11.39	-19.12	21	21	630	7.56	O	Can
Cc70_15CAN	79.00	15-17.5	CC-A1.1	8.49	-16.18	35	3	90	1.08	N	Can
Cc70_15CAN	79.00	15-17.5	CC-A1.1	7.85	-16.83	35	11	330	3.96	O	CV
Cc70_15CAN	79.00	15-17.5	CC-A1.1	8.71	-16.25	35	19	570	6.84	N	Can
Cc70_15CAN	79.00	15-17.5	CC-A1.1	8.37	-17.43	35	27	810	9.72	O	CV
Cc70_15CAN	79.00	15-17.5	CC-A1.1	8.67	-16.39	35	35	1050	12.6	N	Can

Table S3: Populations used in the F statistic and the exact test of populations differentiation.

	Foraging Grounds
CI	Canary Islands, Spain
AZ	Azores, Portugal
MD	Madeira, Madeira
AND	Andalusia, Spain
ALGE	Algerian Sea
CATBAL	Catalan Coast and Balearic Islands
NESP	North Spain
THYR	Thyrrenian sea
ION	Ionian sea
LAMP	Lampedusa, Greece
NADR	North Adriatic
SADR	South Adriatic
SLEV	South Levantine

	Rookeries
CV	Cabo Verde
NUSA	North USA
CEFL	Central East Florida
SEFL	South East Florida
DRSL	Dry Tortugas, Florida and Cay Sal, Bahamas
QRM	Quintana Roo, Mexico and Cuba;
KEY	Keewdin Island, Florida
CSK	Casey Key, Florida
NWFL	Northwestern Florida
NBRAZIL	North Brasil
ESP	Espiritu Santo, Brazil
RIO	Rio de Janeiro, Brazil
CAL	Cagliari, Italia
WGRG	Western Greece
CRT	Creta, Greece
EMED	East Mediterranean (Israel, Lebanon and Cyprus)
LIBYTUN	Libya and Tunisia
DYDL	Dalyan and Dalaman, Turkey
TKW	Western Turkey

Table S4: Atlantic and Mediterranean rookeries population pairwise FSTs.

	CV	NUSA	CEFL	SEFL	DRSL	QRM	KEY	CSK	NWFL	NBRAZIL	ESP	RIO	CAL	WGRG	CRT	EMED	LIBYTUN	DYDL	TKW
CV	0.00																		
NUSA	0.65	0.00																	
CEFL	0.44	0.18	0.00																
SEFL	0.35	0.58	0.24	0.00															
DRSL	0.50	0.95	0.41	0.07	0.00														
QRM	0.30	0.57	0.29	0.07	0.18	0.00													
KEY	0.41	0.36	0.03	0.13	0.30	0.19	0.00												
CSK	0.38	0.21	0.01	0.19	0.36	0.22	0.02	0.00											
NWFL	0.53	0.12	0.06	0.41	0.69	0.41	0.16	0.08	0.00										
NBRAZIL	0.43	0.82	0.46	0.38	0.58	0.30	0.42	0.39	0.61	0.00									
ESP	0.40	0.89	0.43	0.35	0.58	0.26	0.39	0.36	0.61	0.01	0.00								
RIO	0.51	0.92	0.52	0.47	0.72	0.39	0.51	0.46	0.71	0.08	0.15	0.00							
CAL	0.43	0.85	0.37	0.10	0.19	0.14	0.26	0.31	0.60	0.46	0.42	0.59	0.00						
WGRG	0.55	0.94	0.44	0.12	0.01	0.23	0.36	0.40	0.73	0.65	0.67	0.77	0.26	0.00					
CRT	0.48	0.95	0.40	0.07	0.05	0.15	0.28	0.34	0.67	0.52	0.50	0.68	0.16	0.09	0.00				
EMED	0.61	0.92	0.47	0.16	0.02	0.30	0.43	0.45	0.78	0.74	0.77	0.82	0.37	0.01	0.14	0.00			
LIBYTUN	0.42	0.85	0.35	0.06	0.15	0.11	0.24	0.28	0.58	0.44	0.39	0.57	0.15	0.23	0.12	0.31	0.00		
DYDL	0.44	0.83	0.38	0.14	0.31	0.17	0.28	0.30	0.60	0.47	0.44	0.59	0.27	0.39	0.27	0.47	0.17	0.00	
TKW	0.50	0.88	0.40	0.07	0.07	0.17	0.29	0.34	0.66	0.56	0.56	0.68	0.20	0.10	0.09	0.11	0.12	0.16	0.00

Table S5: Atlantic and Mediterranean rookeries matrix of significant Fst P-values.

	CV	NUSA	CEFL	SEFL	DRSL	QRM	KEY	CSK	NWFL	NBRZ	ESP	RIO	CAL	WGRG	CRT	EMED	LIBTUN	DYDL	TKW
CV		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NUSA	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CEFL	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SEFL	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DRSL	+	+	+	+		+	+	+	+	+	+	+	+	-	-	-	+	+	+
QRM	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
KEY	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
CSK	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
NWFL	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
NBRAZIL	+	+	+	+	+	+	+	+	+		-	+	+	+	+	+	+	+	+
ESP	+	+	+	+	+	+	+	+	+	-		+	+	+	+	+	+	+	+
RIO	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
CAL	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
WGRG	+	+	+	+	-	+	+	+	+	+	+	+	+		+	-	+	+	+
CRT	+	+	+	+	-	+	+	+	+	+	+	+	+	+		+	+	+	+
EMED	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+		+	+	+
LIBTUN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+
DYDL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
TKW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Table S6: Atlantic and Mediterranean foraging grounds population pairwise FSTs.

	CI	AZ	MD	AND	ALGE	CATBAL	NESP	THYR	ION	LAMP	NADR	SADR	SLEV
CI	0.00												
AZ	0.00	0.00											
MD	-0.01	-0.01	0.00										
AND	0.01	-0.01	-0.01	0.00									
ALGE	0.04	0.01	0.02	0.00	0.00								
CATBAL	0.23	0.20	0.22	0.17	0.10	0.00							
NESP	0.20	0.16	0.18	0.14	0.07	-0.01	0.00						
THYR	0.22	0.19	0.21	0.16	0.09	-0.02	-0.01	0.00					
ION	0.25	0.23	0.25	0.20	0.14	0.00	0.01	0.01	0.00				
LAMP	0.08	0.05	0.06	0.03	0.00	0.05	0.02	0.04	0.08	0.00			
NADR	0.31	0.30	0.32	0.26	0.20	0.01	0.04	0.02	0.01	0.13	0.00		
SADR	0.34	0.32	0.35	0.28	0.22	0.03	0.05	0.04	0.04	0.15	-0.03	0.00	
SLEV	0.26	0.24	0.26	0.21	0.15	-0.02	0.00	0.00	-0.02	0.08	-0.01	0.01	0.00

Table S7: Atlantic and Mediterranean foraging grounds matrix of significant Fst P-values.

	CI	AZ	MD	AND	ALGE	CATBAL	NESP	THYR	ION	LAMP	NADR	SADR	SLEV
CI		-	-	-	+	+	+	+	+	+	+	+	+
AZ	-		-	-	-	+	+	+	+	+	+	+	+
MD	-	-		-	-	+	+	+	+	+	+	+	+
AND	-	-	-		-	+	+	+	+	+	+	+	+
ALGE	+	-	-	-		+	+	+	+	-	+	+	+
CATBAL	+	+	+	+	+		-	-	-	+	-	-	-
NESP	+	+	+	+	+	-		-	-	+	+	+	-
THYR	+	+	+	+	+	-	-		-	+	-	-	-
ION	+	+	+	+	+	-	-	-		+	-	-	-
LAMP	+	+	+	+	-	+	+	+	+		+	+	+
NADR	+	+	+	+	+	-	+	-	-	+		-	-
SADR	+	+	+	+	+	-	+	-	-	+	-		-
SLEV	+	+	+	+	+	-	-	-	-	+	-	-	

Table S8: Genetic and stable isotope results from Cabo Verdean loggerheads (n=105), including recaptured (R; n=44) and untagged turtles (U; n=60). The sampling year and the isotopic values of δ^{15}N‰ and δ^{13}C‰ of the different studied carapace layers, from the innermost layer to the most external layer (Ext. layer), are shown. The feeding strategy (Stage), neritic (N) or oceanic (O) determined by the stable isotopic results is included. Also the changes in habitat, neritic to oceanic (NO) and oceanic to neritic (ON). Abbreviations: Minimun curve carapace lenght (minCCL).

Turtle	**minCCL** (cm)	**Age** (years)	**Year**	**Haplotype**	**Recaptured**	**δ^{13}C‰ (1)** Inner layer	**δ^{13}C‰ (2)** Ext. Layer	**δ^{15}N‰ (1)** Inner layer	**δ^{15}N‰ (2)** Ext. Layer	**Stage**
Cc3_17CV	87.60	25-32.5	2017	CC-A1.3	R	-17.87	-17.33	10.70	9.63	O
Cc7_17CV	86.30	25-32.5	2017	CC-A1.3	R	-18.26	-16.06	9.95	10.69	NO
Cc8_17CV	86.50	25-32.5	2017	CC-A1.3	R	-19.26	-17.53	8.97	9.52	O
Cc21_17CV	91.20	32.5-40	2017	CC-A1.3	R	-18.61	-19.60	9.09	9.29	O
Cc22_17CV	87.20	25-32.5	2017	CC-A1.3	R	-18.52	-18.28	9.55	9.51	O
Cc23_17CV	87.00	25-32.5	2017	CC-A11.2	R	-17.84	-19.08	10.24	8.85	O
Cc24_17CV	86.50	25-32.5	2017	CC-A1.7	R	-17.84	-18.30	9.93	8.60	O
Cc25_17CV	88.00	25-32.5	2017	CC-A17.1	R	-18.13	-19.29	9.51	9.38	O
Cc28_17CV	88.50	25-32.5	2017	CC-A1.3	R	-17.41	-18.02	10.45	7.95	O
Cc29_17CV	88.20	25-32.5	2017	CC-A17.1	R	-17.18	-16.82	10.81	10.14	NO
Cc30_17CV	87.00	25-32.5	2017	CC-A17.1	R	-16.87	-18.68	10.94	8.90	ON
Cc31_17CV	85.80	25-32.5	2017	CC-A1.3	R	-17.04	-16.80	10.73	10.80	NO
Cc32_17CV	85.80	25-32.5	2017	CC-A17.1	R	-16.81	-18.28	9.84	9.99	ON
Cc33_17CV	85.80	25-32.5	2017	CC-A1.3	R	-18.33	-14.08	9.55	12.82	NO
Cc34_17CV	87.00	25-32.5	2017	CC-A17.1	R	-18.22	-17.64	9.45	9.54	O

Cc35_17CV	88.50	25-32.5	2017	CC-A17.1	R	-17.56	-17.02	10.67	9.75	O
Cc36_17CV	86.50	25-32.5	2017	CC-A17.1	R	-17.89	-16.96	9.43	9.37	NO
Cc37_17CV	87.00	25-32.5	2017	CC-A1.3	R	-18.15	-20.20	10.10	8.43	O
Cc38_17CV	86.00	25-32.5	2017	CC-A17.1	R	-17.51	-17.99	10.82	9.18	O
Cc39_17CV	88.00	25-32.5	2017	CC-A1.3	R	-17.56	-17.86	10.98	9.55	O
Cc40_17CV	87.00	25-32.5	2017	CC-A1.3	R	-16.83	-15.59	12.07	11.83	N
Cc41_17CV	86.20	25-32.5	2017	CC-A1.3	R	-15.96	-18.74	12.40	8.42	ON
Cc42_17CV	86.50	25-32.5	2017	CC-A1.3	R	-18.51	-17.81	9.92	9.74	O
Cc43_17CV	92.20	32.5-40	2017	CC-A1.3	R	-16.07	-15.85	12.71	12.81	N
Cc45_17CV	86.00	25-32.5	2017	CC-A1.4	R	-17.96	-19.10	9.32	8.80	O
Cc46_17CV	86.00	25-32.5	2017	CC-A17.1	R	-17.72	-17.92	9.40	9.04	O
Cc47_17CV	86.00	25-32.5	2017	CC-A17.1	R	-17.53	-19.59	10.12	8.31	O
Cc48_17CV	87.00	25-32.5	2017	CC-A17.1	R	-18.49	-19.88	9.74	7.91	O
Cc49_17CV	86.00	25-32.5	2017	CC-A1.3	R	-16.52	-16.42	12.71	11.75	N
Cc50_17CV	86.00	25-32.5	2017	CC-A2.1	R	-15.92	-15.45	11.07	11.30	N
Cc51_17CV	86.00	25-32.5	2017	CC-A1.3	R	-18.03	-17.53	9.97	9.83	O
Cc52_17CV	86.00	25-32.5	2017	CC-A17.1	R	-17.63	-17.16	10.83	10.61	O
Cc54_17CV	91.00	32.5-40	2017	CC-A1.3	R	-18.31	-19.20	9.18	8.36	O
Cc56_17CV	86.30	25-32.5	2017	CC-A1.7	R	-16.92	-17.81	11.81	10.45	ON
Cc57_17CV	86.60	25-32.5	2017	CC-A1.3	R	-17.99	-18.11	10.12	10.14	O
Cc58_17CV	92.00	32.5-40	2017	CC-A1.4	R	-18.02	-17.81	10.40	9.88	O
Cc05_15CV	86.00	25-32.5	2015	CC-A17.1	R	-16.93	-20.29	11.39	10.22	ON

Cc16_15CV	92.50	32.5-40	2015	CC-A1.3	R	-14.98	-14.98	14.06	13.75	N
Cc29_15CV	86.00	25-32.5	2015	CC-A17.1	R	-17.51	-16.74	10.28	10.81	ONO
Cc36_15CV	89.00	25-32.5	2015	CC-A1.3	R	-16.12	-15.52	13.01	12.62	N
Cc39_15CV	88.00	25-32.5	2015	CC-A11.6	R	-18.42	-18.30	10.09	10.32	O
Cc40_15CV	89.00	25-32.5	2015	CC-A17.1	R	-17.18	-17.55	12.29	12.22	O
Cc45_15CV	94	32.5-40	2015	CC-A1.3	R	-14.68	-13.86	15.05	13.69	N
Cc47_15CV	86.00	25-32.5	2015	CC-A1.3	R	-18.86	-17.99	8.78	9.49	O
Cc01_17CV	90.80	32.5-40	2017	CC-A1.3	U	-17.72	-16.65	11.72	12.45	NO
Cc02_17CV	85.00	25-32.5	2017	CC-A1.4	U	-17.93	-18.96	9.47	8.82	O
Cc04_17CV	86.60	25-32.5	2017	CC-A1.3	U	-17.31	-19.04	10.09	9.61	O
Cc05_17CV	88.00	25-32.5	2017	CC-A1.3	U	-17.51	-17.73	10.00	9.07	O
Cc06_17CV	88.00	25-32.5	2017	CC-A1.3	U	-17.09	-15.25	10.22	13.38	NO
Cc09_17CV	87.00	25-32.5	2017	CC-A1.3	U	-18.00	-18.23	9.86	9.91	O
Cc10_17CV	84.50	20-25.5	2017	CC-A17.2	U	-18.23	-18.16	9.69	9.20	O
Cc11_17CV	85.50	25-32.5	2017	CC-A1.3	U	-15.59	-15.71	13.53	12.46	N
Cc12_17CV	86.20	25-32.5	2017	CC-A1.3	U	-17.51	-17.31	10.88	10.18	O
Cc13_17CV	83.80	20-25.5	2017	CC-A17.1	U	-18.21	-18.22	10.33	9.41	O
Cc14_17CV	93.00	32.5-40	2017	CC-A1.3	U	-15.45	-15.45	14.27	14.24	N
Cc15_17CV	85.50	25-32.5	2017	CC-A17.1	U	-17.93	-15.89	10.58	10.70	NO
Cc16_17CV	84.80	20-25.5	2017	CC-A17.1	U	-18.24	-17.81	9.25	9.58	O
Cc17_17CV	86.30	25-32.5	2017	CC-A1.3	U	-17.90	-16.96	11.45	12.20	NO
Cc18_17CV	91.50	32.5-40	2017	CC-A1.3	U	-17.09	-16.36	16.15	15.23	NO

Cc19_17CV	103.80	>40	2017	CC-A17.1	U	-14.85	-14.58	13.52	12.45	N
Cc20_17CV	86.20	25-32.5	2017	CC-A1.3	U	-18.00	-17.24	8.86	10.32	O
Cc27_17CV	93.00	32.5-40	2017	CC-A17.1	U	-16.59	-16.46	11.37	10.66	N
Cc01_15CV	79.00	17.5-20	2015	CC-A1.3	U	-18.72	-18.14	10.35	9.79	O
Cc02_15CV	87.50	25-32.5	2015	CC-A1.3	U	-17.12	-16.70	12.08	11.40	NO
Cc03_15CV	79.00	17.5-20	2015	CC-A1.3	U	-18.52	-19.02	10.15	8.12	O
Cc04_15CV	80.00	17.5-20	2015	CC-A17.1	U	-17.40	-18.30	10.50	9.69	O
Cc06_15CV	89.00	25-32.5	2015	CC-A1.3	U	-17.98	-18.40	10.03	8.42	O
Cc07_15CV	85.50	25-32.5	2015	CC-A1.3	U	-16.43	-15.60	13.08	13.14	NO
Cc08_15CV	81.50	20-25.5	2015	CC-A17.1	U	-19.55	-18.28	8.46	9.31	O
Cc09_15CV	81.00	20-25.5	2015	CC-A1.3	U	-18.50	-18.12	9.50	10.43	O
Cc10_15CV	80.50	20-25.5	2015	CC-A2.1	U	-18.38	-17.40	10.65	10.31	O
Cc11_15CV	78.00	17.5-20	2015	CC-A1.3	U	-19.24	-19.19	8.41	7.25	O
Cc12_15CV	84.50	20-25.5	2015	CC-A17.1	U	-17.83	-18.25	10.56	8.61	O
Cc13_15CV	81.00	20-25.5	2015	CC-A1.3	U	-17.41	-18.00	10.28	9.69	O
Cc14_15CV	96.00	>40	2015	CC-A1.3	U	-16.39	-16.35	11.26	9.98	N
Cc15_15CV	85.00	25-32.5	2015	CC-A1.3	U	-18.44	-18.79	10.27	9.19	O
Cc17_15CV	76.00	17.5-20	2015	CC-A1.3	U	-18.28	-19.33	10.08	8.99	O
Cc18_15CV	84.00	20-25.5	2015	CC-A1.3	U	-18.06	-19.69	9.85	9.26	O
Cc19_15CV	83.50	20-25.5	2015	CC-A1.7	U	-18.13	-21.21	10.00	7.76	O
Cc20_15CV	77.00	17.5-20	2015	CC-A1.7	U	-17.97	-18.30	10.71	9.63	O
Cc21_15CV	84.50	20-25.5	2015	CC-A1.4	U	-17.49	-17.00	10.92	11.21	O

Cc22_15CV	85.00	25-32.5	2015	CC-A1.3	U	-19.11	-19.39	10.20	7.83	O
Cc23_15CV	81.50	20-25.5	2015	CC-A1.3	U	-17.52	-18.10	10.33	9.44	O
Cc24_15CV	82.00	20-25.5	2015	CC-A17.1	U	-17.49	-17.75	9.90	9.30	O
Cc25_15CV	94.00	32.5-40	2015	CC-A1.3	U	-17.12	-16.46	11.87	11.21	NO
Cc26_15CV	77.50	17.5-20	2015	CC-A1.3	U	-17.60	-18.62	10.38	9.06	O
Cc27_15CV	81.00	20-25.5	2015	CC-A17.1	U	-18.52	-17.77	9.78	10.94	O
Cc28_15CV	81.50	20-25.5	2015	CC-A17.1	U	-17.53	-18.16	10.47	10.67	O
Cc30_15CV	92.00	32.5-40	2015	CC-A1.3	U	-17.49	-17.39	11.99	10.91	O
Cc31_15CV	79.00	17.5-20	2015	CC-A1.3	U	-18.59	-17.54	9.66	10.02	O
Cc32_15CV	75.00	17.5-20	2015	CC-A17.1	U	-17.91	-17.87	10.97	9.93	O
Cc33_15CV	86.00	25-32.5	2015	CC-A17.1	U	-15.56	-16.76	13.10	11.77	N
Cc34_15CV	84.00	20-25.5	2015	CC-A1.3	U	-18.03	-17.40	10.76	9.63	O
Cc35_15CV	81.00	20-25.5	2015	CC-A1.7	U	-17.97	-17.02	10.43	10.30	O
Cc37_15CV	94	32.5-40	2015	CC-A1.4	U	-15.93	-16.24	11.35	11.64	N
Cc38_15CV	85.50	25-32.5	2015	CC-A17.1	U	-18.35	-19.28	9.96	7.99	O
Cc41_15CV	82.00	20-25.5	2015	CC-A1.3	U	-17.69	-19.67	10.89	10.00	O
Cc43_15CV	88.00	25-32.5	2015	CC-A1.3	U	-18.50	-18.57	11.29	9.89	O
Cc44_15CV	83.50	20-25.5	2015	CC-A1.3	U	-16.00	-18.06	11.44	10.81	ON
Cc46_15CV	87.00	25-32.5	2015	CC-A1.3	U	-17.81	-18.01	10.75	9.31	O
Cc48_15CV	85.50	25-32.5	2015	CC-A1.3	U	-18.56	-18.13	10.10	9.67	O
Cc49_15CV	89.00	25-32.5	2015	CC-A1.3	U	-14.49	-15.19	14.96	14.23	N
Cc50_15CV	83.50	20-25.5	2015	CC-A17.1	U	-17.11	-18.67	9.94	8.80	O

Cc72_15CV	84.00	20-25.5	2015	CC-A17.1	U	-18.38	-18.58	11.13	9.64	O
Cc73_15CV	83.50	20-25.5	2015	CC-A17.1	U	-18.05	-18.93	10.19	8.54	O

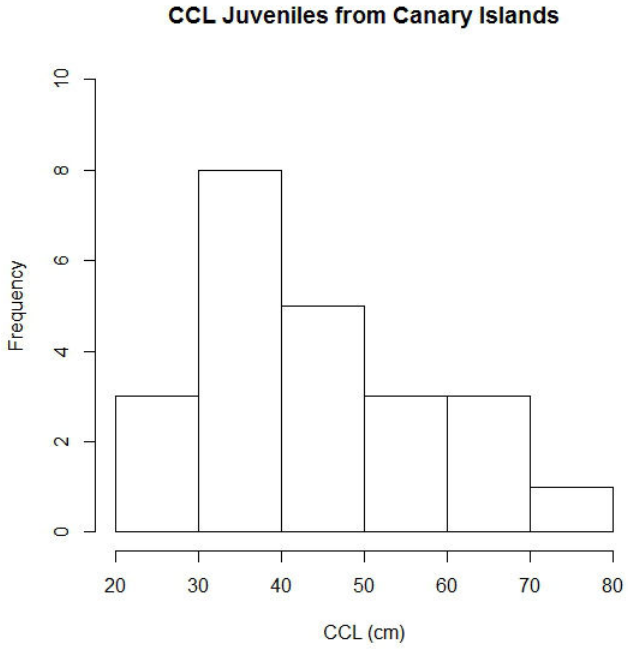
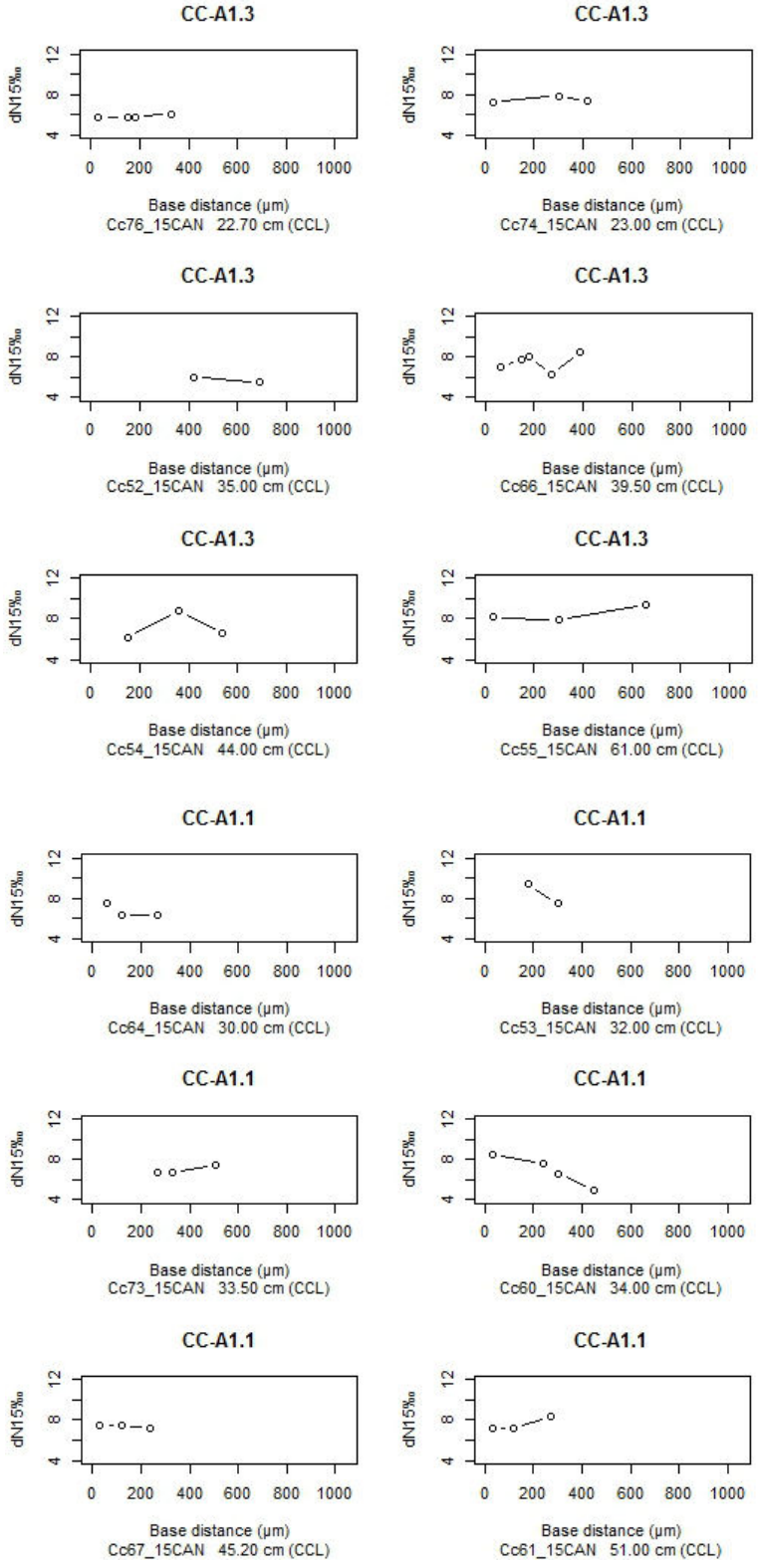


Figure S1: Curve carapace length (CCL) of juveniles from Canary Islands.


CC-A1.3
dN15‰
Base distance (µm)
Cc76_15CAN 22.70 cm (CCL)
CC-A1.3
Base distance (µm)
Cc74_15CAN 23.00 cm (CCL)
CC-A1.3
Base distance (µm)
Cc52_15CAN 35.00 cm (CCL)
CC-A1.3
Base distance (µm)
Cc66_15CAN 39.50 cm (CCL)
CC-A1.3
Base distance (µm)
Cc54_15CAN 44.00 cm (CCL)
CC-A1.3
Base distance (µm)
Cc55_15CAN 61.00 cm (CCL)
CC-A1.1
Base distance (µm)
Cc64_15CAN 30.00 cm (CCL)
CC-A1.1
Base distance (µm)
Cc53_15CAN 32.00 cm (CCL)
CC-A1.1
Base distance (µm)
Cc73_15CAN 33.50 cm (CCL)
CC-A1.1
Base distance (µm)
Cc60_15CAN 34.00 cm (CCL)
CC-A1.1
Base distance (µm)
Cc67_15CAN 45.20 cm (CCL)
CC-A1.1
Base distance (µm)
Cc61_15CAN 51.00 cm (CCL)

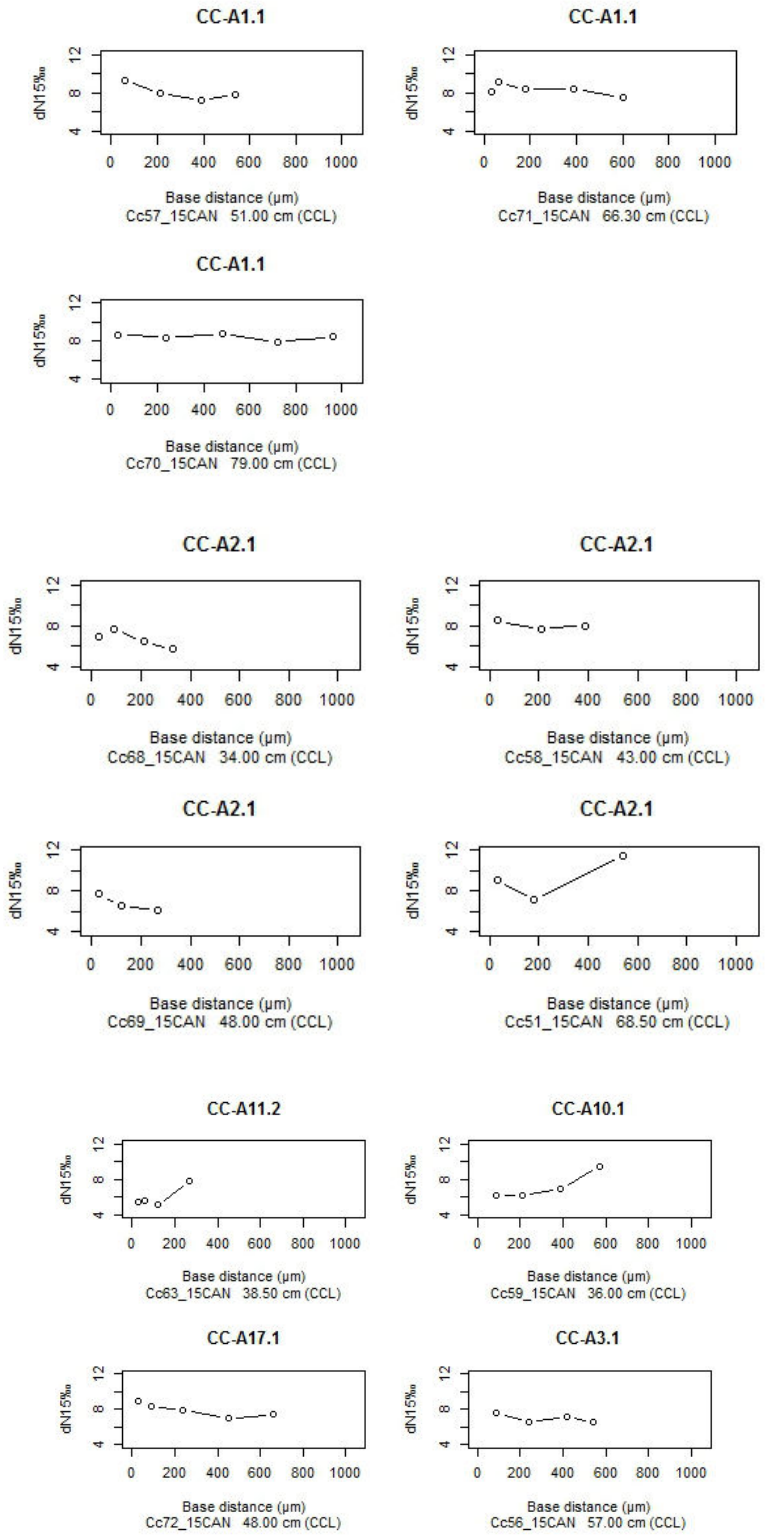
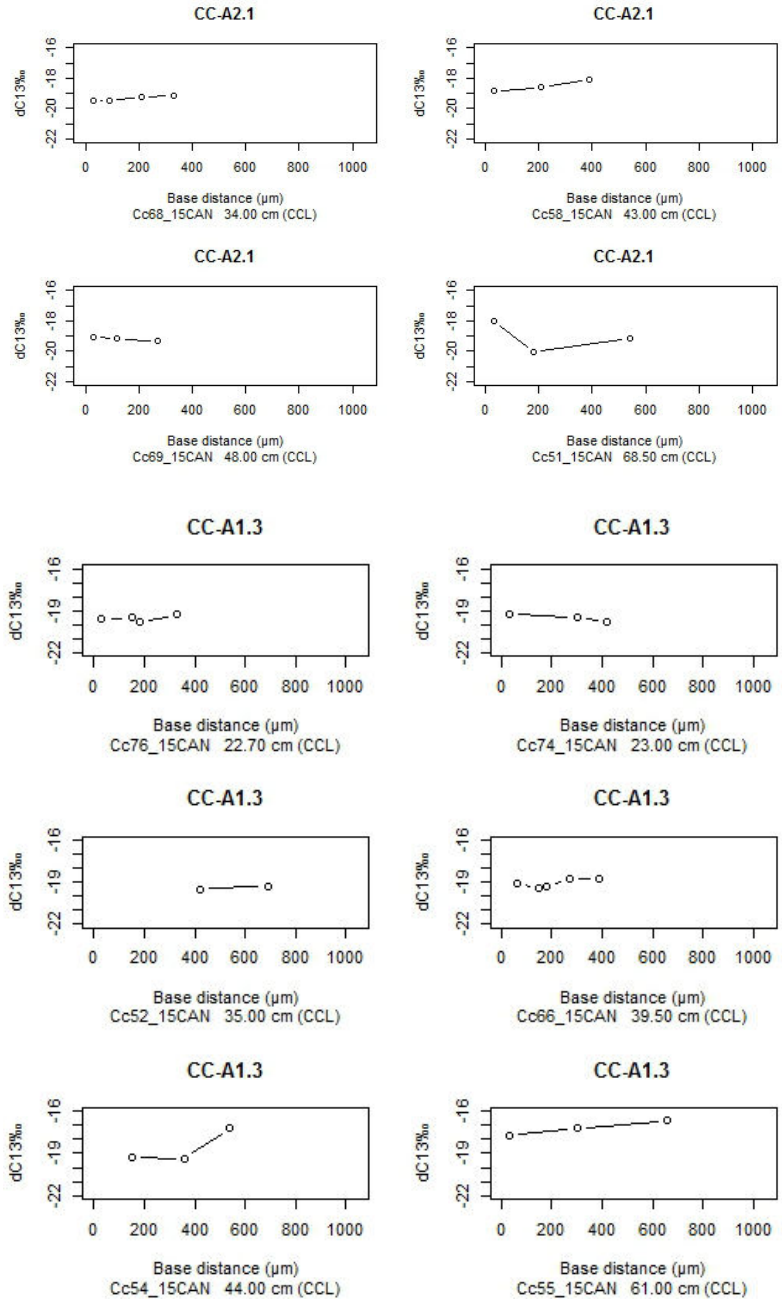


Figure S2: δ^{15}N‰ values of every juvenile depending on base distance, from the innermost layer.


CC-A2.1
dC13‰
Base distance (µm)
Cc68_15CAN 34.00 cm (CCL)
CC-A2.1
dC13‰
Base distance (µm)
Cc58_15CAN 43.00 cm (CCL)
CC-A2.1
dC13‰
Base distance (µm)
Cc69_15CAN 48.00 cm (CCL)
CC-A2.1
dC13‰
Base distance (µm)
Cc51_15CAN 68.50 cm (CCL)
CC-A1.3
dC13‰
Base distance (µm)
Cc76_15CAN 22.70 cm (CCL)
CC-A1.3
dC13‰
Base distance (µm)
Cc74_15CAN 23.00 cm (CCL)
CC-A1.3
dC13‰
Base distance (µm)
Cc52_15CAN 35.00 cm (CCL)
CC-A1.3
dC13‰
Base distance (µm)
Cc66_15CAN 39.50 cm (CCL)
CC-A1.3
dC13‰
Base distance (µm)
Cc54_15CAN 44.00 cm (CCL)
CC-A1.3
dC13‰
Base distance (µm)
Cc55_15CAN 61.00 cm (CCL)

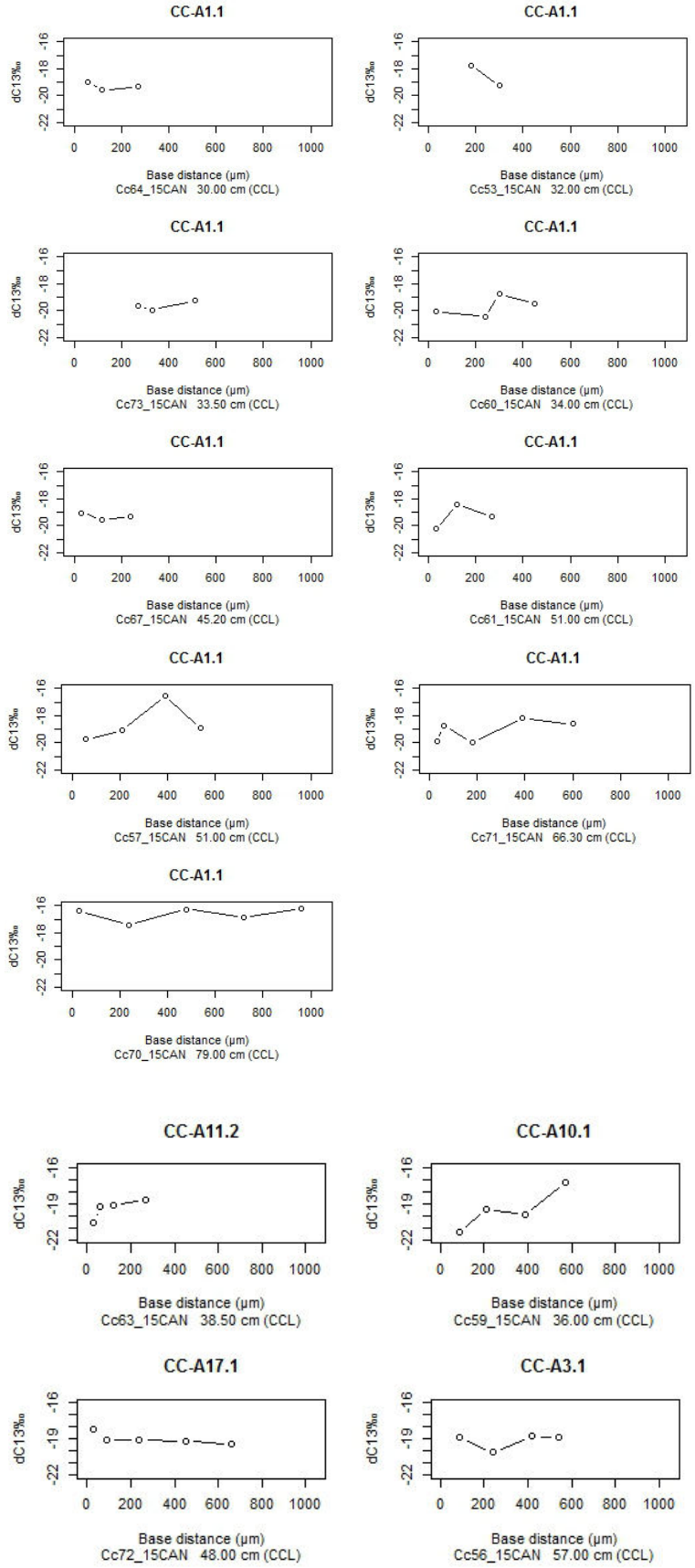
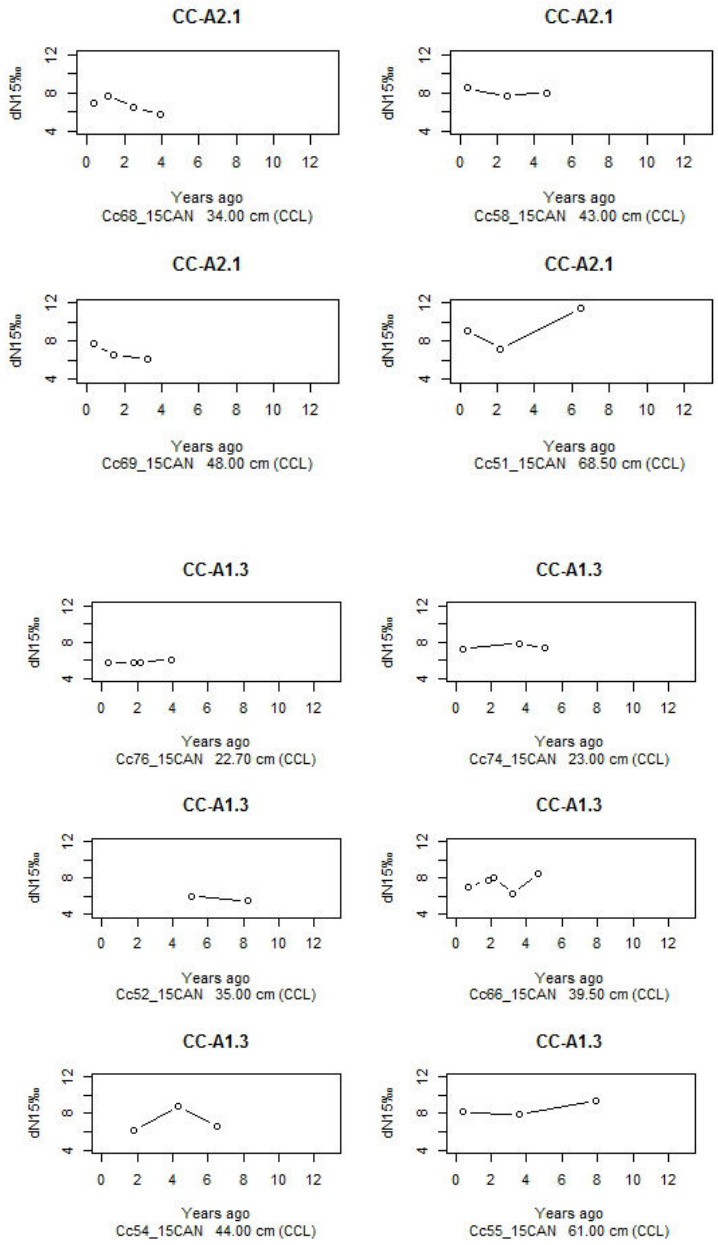


Figure S3: $\delta^{13}C‰$ values of every depending on base distance, from the innermost layer.


CC-A2.1
dN15‰
Years ago
Cc68_15CAN 34.00 cm (CCL)
CC-A2.1
dN15‰
Years ago
Cc58_15CAN 43.00 cm (CCL)
CC-A2.1
dN15‰
Years ago
Cc69_15CAN 48.00 cm (CCL)
CC-A2.1
dN15‰
Years ago
Cc51_15CAN 68.50 cm (CCL)
CC-A1.3
dN15‰
Years ago
Cc76_15CAN 22.70 cm (CCL)
CC-A1.3
dN15‰
Years ago
Cc74_15CAN 23.00 cm (CCL)
CC-A1.3
dN15‰
Years ago
Cc52_15CAN 35.00 cm (CCL)
CC-A1.3
dN15‰
Years ago
Cc66_15CAN 39.50 cm (CCL)
CC-A1.3
dN15‰
Years ago
Cc54_15CAN 44.00 cm (CCL)
CC-A1.3
dN15‰
Years ago
Cc55_15CAN 61.00 cm (CCL)

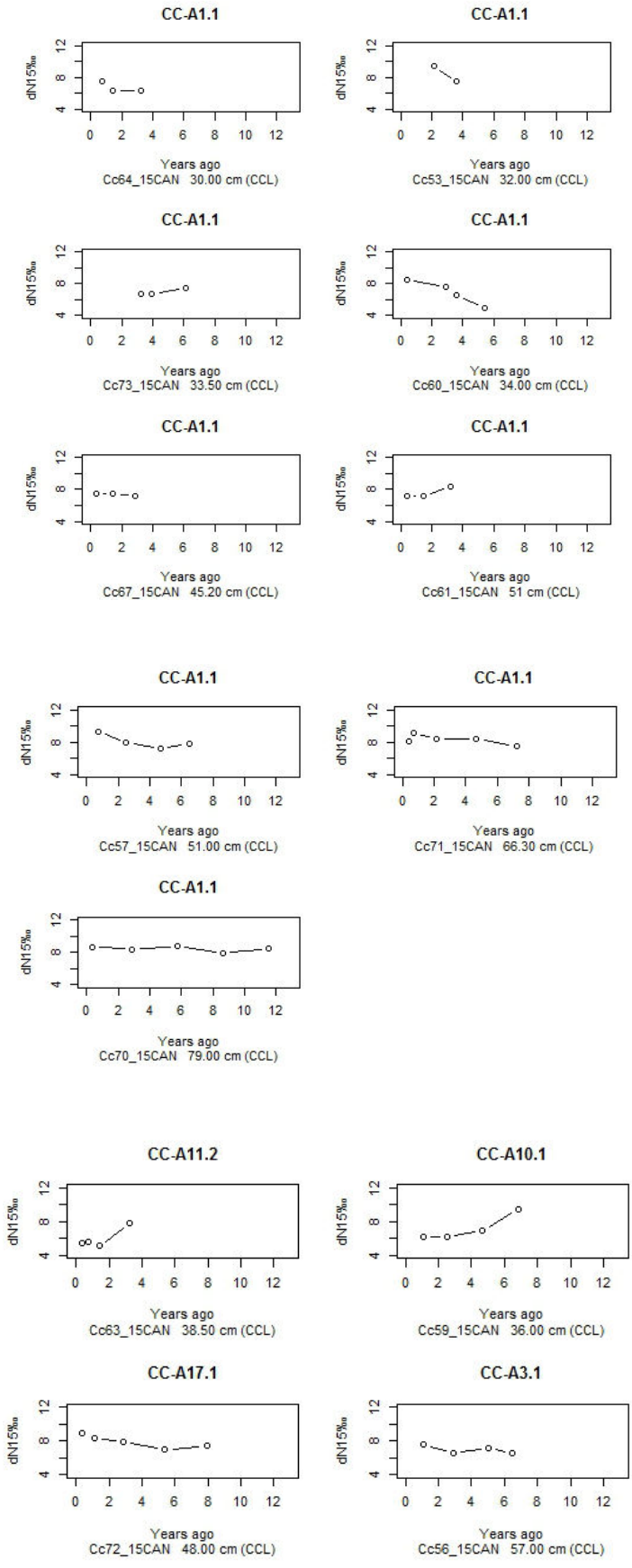
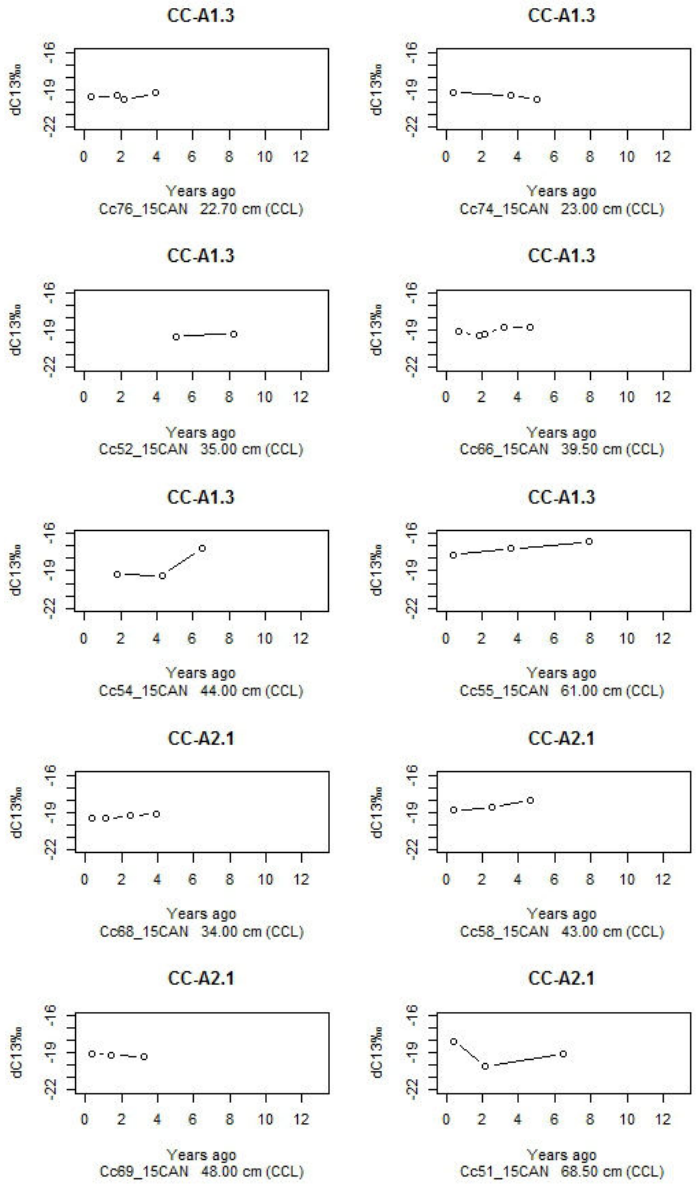


Figure S4: δ^{15}N‰ values of carapace on time (years ago since its formation) for juveniles.


CC-A1.3
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc76_15CAN 22.70 cm (CCL)
CC-A1.3
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc74_15CAN 23.00 cm (CCL)
CC-A1.3
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc52_15CAN 35.00 cm (CCL)
CC-A1.3
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc66_15CAN 39.50 cm (CCL)
CC-A1.3
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc54_15CAN 44.00 cm (CCL)
CC-A1.3
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc55_15CAN 61.00 cm (CCL)
CC-A2.1
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc68_15CAN 34.00 cm (CCL)
CC-A2.1
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc58_15CAN 43.00 cm (CCL)
CC-A2.1
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc69_15CAN 48.00 cm (CCL)
CC-A2.1
dC13‰
-16
-19
-22
0 2 4 6 8 10 12
Years ago
Cc51_15CAN 68.50 cm (CCL)

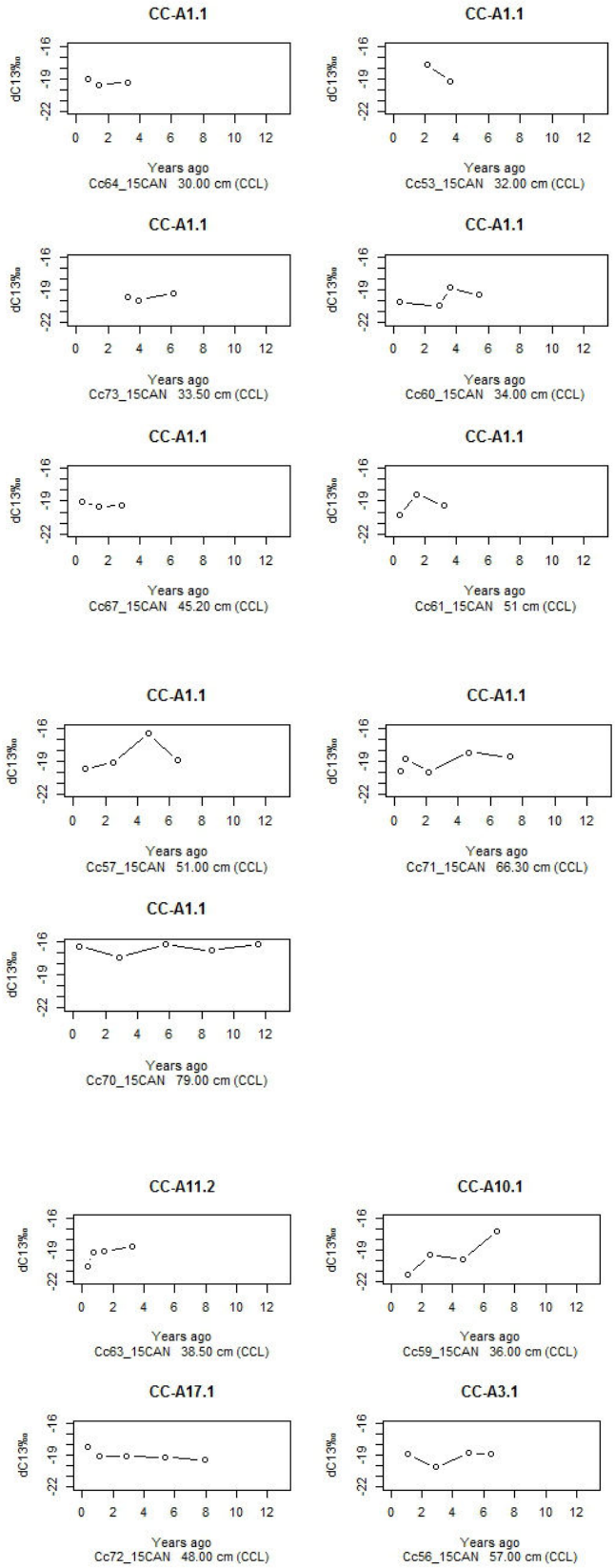


Figure S5: $\delta^{13}C‰$ values of carapace on time (years ago years ago since its formation) for juveniles.

Evaluation

Developed activities during the elaboration of the Project.

During the development of the project the different tasks were conducted, in first place, organize de blood samples from 2015 and 2017. Then, DNA was extracted from these blood samples using the E.Z.N.A.® Blood DNA Mini Kit (OMEGA bio-tek) following manufacturer's protocols. After that, extracted DNA was quantify with the Thermo Scientific NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) before undergo PCR reactions. PCR reactions have to be adjusted before finding the ideal concentrations and volumes of the PCR mixture reactives. For analyzing the PCR reaction an agarose gel electrophoresis was performed. The concentrations for the electrophoresis gel were also tested several times for finding the most adequate concentration. Finally, successful PCR products were sent to Secugen (Secugen S.L., Spain) to be purified and sequenced. After receiving the results of the sequenced samples, the ones with enough quality were aligned using the BioEdit Sequence Alignment Editor software version 7.2.6.1 (Copyright© 1997-2017, Hall 1999). The obtained sequences were classified according to standardized nomenclature. The analysis of the genetic results was performed with Arlequin v.3.0 (Excoffier et al. 2005), Bayes and R-Project statistical software.
Stable isotopes samples were processed at *Centres Científics i Tecnològics de la Universitat de Barcelona.* Results were received in May, December and April and the statistical analysis was performed using R-Project statistical software.

Trainig received

The development of this project brings me the opportunity to learn more about genetics and specially about marine turtles, besides the main techniques and protocols for extracting and analyzing mitochondrial DNA. Moreover, I was taught how to use different hardware such as NanoDrop, and different software such as BioEdit and Arlequin. Furthermore, I was showed how to perform different analysis with a variety of statistical packages in R-Project.

Level of integration and implication in the department

All the laboratory procedures where developed in the Parque Científico Técnico and in the Servicio de acuicultura y biotecnología de alta especialización (SABE) in Gran Canaria. I was help and encouraged every moment for the lab technicians, the rest of colleagues and especially from my tutor, who provided me information and proposed me projects and activities.

Most significant positive and negative aspects of the project's development

The most positive aspect of this project was the opportunity of learning, and being taught, about genetics and stable isotopes, tools that I have never had the opportunity to work before. Moreover, it has been an enriching experience to develop it in the SABE infrastructures and being surrounded by professionals from different fields.

As a negative aspect I only highlight the difficulty of having done the master's practices in another country, doing an internship in another research project. For this reason, I had not all the time I would have liked to develop and write the project. However, I am really grateful for the Erasmus grant that allowed me to go to the university of Genova and for the confidence my project's tutor had on me.

Personal valuation of achieved learning

As I said before, developing the Project with mi tutor in the Ecoaqua group has been a great experience that introduced me to new techniques in the field of stable isotopes and genetics that were completely new for me. I am really grateful for all the help, patience, advice and confidence my tutor Caty, the team and technicians have provided me.