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Different indicators of fitness of the sea turtle (*Lepidochelys olivacea*): Gulf of Guinea as a study case

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Executive summary

The olive ridley sea turtle (*Lepidochelys olivacea*) has one of the biggest populations of sea turtles in the Gulf of Guinea. Once the females spend high amounts of energy in migrations from foraging to spawning areas during the breeding season, it is expected that their physiological condition is impaired. The assessment of populations' health using demographic parameters, such as individual growth rate, is important to determine the status and viability of them. Thus, the aim of this research was to analyse the body condition of *L. olivacea* females and hatchlings during the nesting season using morphometric and behavioral data. Complementarily, we also aimed to assess their physiological condition and growth rate, through a series of biochemical indices based on nucleic acid concentrations, and their trophic level position by analyzing the skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ content. In fact, ecophysiological indicators such as RNA:DNA (sRD) and stable isotopes ratios are common methods for the fitness and feeding ecology study of nesting females, depending on the environmental conditions, such as temperature and food availability. We analyzed 435 skin samples for physiological condition and 33 samples for feeding ecology determination, through fluorescence and mass spectrophotometry, respectively. Overall, results showed that the physiological condition of nesting females was not influenced by the season and the stable isotope analysis revealed that most of the individuals were feeding on the pelagic realm, but some were also feeding on nearshore food items. The physiological condition of hatchlings showed an independence from the females' condition, but incubation temperature had an influence on the reproductive success and in the self-righting ability. The tests of behavioral response also showed correlation with the incubation time, showing that higher periods of incubation will result in faster hatchlings. In conclusion, biochemical indices are efficient tools to measure the condition of sea turtles and combined with different morphometric and behavioral parameters, represent a non-invasive technique for the assessment of potential survival and growth of sea turtles. Available data suggests that even if *L. olivacea* displays a different behavior to other Atlantic turtle species, the purpose is always to achieve the maximum fitness, a trait that was preserved along species radiation and evolution.

Abstract

During the nesting season, *Lepidochelys olivacea* females spend high amounts of energy in migrations from foraging to spawning areas, dumping their reserves. The assessment of populations' health using demographic parameters, such as individual growth rate, is important to determine the status and viability of populations. Thus, the aim of this research was to predict the body condition of *L. olivacea* females and hatchlings during the nesting season using morphometric and behavioral data. Complementarily, we also aimed to assess their physiological condition and feeding ecology, through RNA/DNA ratio and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ content. It was hypothesized that females' condition would decrease along the season and hatchlings condition would depend on the females. Results were not significant neither for females nor for hatchlings, meaning that both showed an independent good condition during all the season. However, the incubation time and temperature showed to be determinant in hatchlings behavior. The stable isotope analysis revealed that the individuals had a pelagic feeding behavior, although some were feeding nearshore. The main conclusion of this study was that the combination of different tools to measure the fitness of sea turtles using minimally invasive techniques, such as morphometric, behavioral and biochemical parameters, showed to be very powerful,.

Keywords: olive ridley sea turtle, RNA:DNA ratio, stable isotopes, megafauna, physiological condition, feeding ecology, behavior ecology

Introduction & Aims

Worldwide, six of the seven sea turtle species are classified as threatened or endangered due to human actions and lifestyles. The major impacts are a result of constant fishing (intentional or accidental) (Lutcavage *et al.*, 1997; Rebecca *et al.*, 2004; Tomás *et al.*, 2008), long-term egg poaching and adults harvesting on nesting beaches for their meat (Koach *et al.*, 2006), marine pollution (Bugoni *et al.*, 2001), global climate change (Hays *et al.*, 2003; Harley *et al.*, 2006 and Witt *et al.*, 2010) and habitat loss (Orth *et al.*, 2006). Therefore, is important to assess the conservation status of wild populations to understand how their conservation management can be improved, avoiding possible episodes of extinction. Gaining information on fitness, growth rates, reproduction potential and health status of hatchlings is fundamental to quantify demographic parameters (Roark *et al.*, 2009; Hawkes *et al.*, 2013) and implement adequate management strategies.

In the “Origin of species“, Darwin introduced the concept of fitness, even if he did not use this word: “I use the term Struggle for Existence in a large and metaphorical sense, including dependence of one being on another, and including not only the life of the individual, but success in leaving progeny”. Biological fitness, also called Darwinian fitness, means the ability to survive to reproductive age, find a mate, and produce offspring. Basically, the more offspring an organism produces during its lifetime, the greater its biological fitness. Given that differences in survival and number of offspring produced depend mainly on an individual's DNA, biological fitness is usually discussed in terms of most and least successful genes, or characteristics. The solution of contingent problems, linked to the survival of the individuals, is useless if they are unable to reproduce, and the two pressures, one for mere survival and one for reproduction, act in synergy and fuel each other (Boero, 2015). Condition indices not only measure the potential survival of an individual in nature as they are also estimators of the future ability to reproduce.

Biochemical indices are being used worldwide to assess organism condition or fitness reflecting the environment quality, regarding a natural context (Dahlhoff, 2004; Gilliers *et al.*, 2004; Amaral *et al.*, 2008). The individual fitness often changes due to the environment variability such as temperature and food availability (Chícharo and Chícharo, 2008), affecting the performance and potential survival of those organisms.

Nucleic acid content and stable isotope ratios are important indicators of the organism fitness and its relation to food sources. The RNA:DNA ratio (also known as sRD - standardized RNA/DNA ratio) has shown to be very useful as an indicator of body condition in a wide range of marine organisms, as invertebrates and fish (Heztel and Wright, 1983; Gilliers *et al.*, 2004; Amaral *et al.*, 2008), providing information about the health status as well as instantaneous growth rates of a population (Vieira *et al.*, 2014), through a minimally-invasive techniques in wild animals.

The RNA content is related to the synthesis of new beneficial proteins and growth and reproduction metabolic rates (Amaral *et al.*, 2009). As RNA content changes along the life cycle of individuals due to protein synthesis, the DNA content, that stores the genetic information, remains constant within a cell (Chicharo *et al.*, 2003). This allows the increase of RNA:DNA ratio when the environmental conditions are favorable (Dahlhoff, 2004) and results in low RNA:DNA for slow growth poorly nourished individuals (Buckley *et al.*, 2008; Ikeda *et al.*, 2007) and with limited potential of reach adult stage or reproduce successful to produce healthy progeny.

Stable isotopes ratios are an important tool in animal feeding ecology, more specifically in the study of individuals' diet (Hobson and Clark, 1992; Angerbjorn *et al.*, 1994; Koch *et al.*, 1995), trophic position and body condition (Gannes *et al.*, 1997). The analysis of this index has been used worldwide in studies of feeding habits of major marine megafauna taxa (see review by Michener & Schell, 1994), as marine mammals (Ruiz-Cooley *et al.*, 2004, Kurle, 2002), seabirds (Hobson 1993), sharks (Estrada *et al.*, 2003), and sea turtles (Godley *et al.*, 1998), mainly through the quantification of natural abundances of carbon (C) and nitrogen (N) stable isotopes of the individual and their resources. Every living being is derived from the atoms that are present in their food (Pasquaud *et al.*, 2008), which allows the determination of the food sources that have been assimilated by the consumers tissue over time through stable isotopic analysis (Caut *et al.*; 2008; Pasquaud *et al.*; 2008; Lebreton *et al.*, 2012). The nitrogen isotope can be especially useful to obtain information about the trophic level of omnivorous species (Bearhop *et al.*, 2004), indicating intraspecific variability, whilst carbon isotopes identifies and quantifies the sources of organic matter regarding mainly the primary producers (Pasquaud *et al.*, 2008), with low variable values along the food web (Vanderklift and Ponsard, 2003).

Although sea turtles are considered a well-studied taxa within the marine megafauna, there are still gaps on sea turtle biology and ecology research especially due to the lack of key data on turtle biology, population status, anthropogenic interactions and environmental hazards (e.g. Bjorndal 1999, Amorocho 2002).

In sea turtles, the individual body size is a simple but important parameter because it is associated with their ecology (Schmidt-Nielsen, 1984) as a result of their maternal contribution, initial size at hatching, growth rate and age at maturity (Congdon and van Loben Sels, 1991). As they are long-lived vertebrates and migrate long distances, their growth rate is frequently determined by mark and recapture programs (Roark *et al.*, 2009), with very low probabilities of recapture (Limpus, 1992) and through repeatedly populations' sampling for use in length-frequency analyses (Bjorndal *et al.*, 1995) which can be extremely exhaustive. Moreover, morphometric measurements in one-year-old individuals, that is the interval that it is considered usable, can induce cumulative changes from long-term growth, that do not correspond to the biochemical index for short-term

growth (within days to weeks), due to different responses to environmental conditions (Ferron and Leggett 1994, Gilliers *et al.*, 2004). Thus, establishing techniques to supplement mark-recapture programs and length-frequency analyses for estimating individual growth rates and nutritional status of sea turtles would substantially improve our ability to evaluate the status of sea turtle populations and its viability on a shorter timescale.

The olive ridley sea turtle, *Lepidochelys olivacea* (Eschscholtz, 1829), is considered the most abundant species of sea turtle in the world (Eguchi *et al.*, 2007), and they are globally distributed in the tropical regions of the South Atlantic, Pacific, and Indian Oceans (Withing *et al.*, 2007b, NOAA Fisheries, 2014). However, human exploitation of this species over the last centuries has led to dramatic population declines in several regions of the world (Vives and Pironio, 1998; Shanker *et al.*, 2004). Olive ridley sea turtles are highly migratory species that have a complex life cycle, with changes in their diet and habits over their life (Reis *et al.*, 2010). They often migrate great distances between feeding and breeding grounds (Limpus *et al.*, 1992) and they expend a considerable energy on this process. A female sea turtle nests on average every 14 days during the breeding season (Kalb, 1999), returning to breed 1 to 3 times on each season (Pritchard and Plotkin 1995), every 2 to 4 years, because the energetic cost of breeding does not allow it to do it more frequently (Plotkin and Bernardo, 2003). Their embryos will survive according to their nutritional condition that depends on the amount and quality of energy reserves (i.e., yolk and albumen) provided by the female (Perrault *et al.*, 2012). The reproductive success may be affected by a variety of factors, among others, the intrinsic maternal reproductive condition, and extrinsic factors, such as temperature, humidity, salinity, levels of respiratory gasses, beach erosion and natural predators (Stokes *et al.*, 2006).

The life cycle of this species comprises neritic and/or oceanic feeding habits that depend on the existence of profitable resources for different life stages (Bolten, 2003), showing a high habitat plasticity (McMahon *et al.*, 2007). The knowledge about the olive ridley sea turtle's feeding and behavioral ecology is very vague and very difficult to determine due to the limited access to their foraging and feeding areas. In this case, the stable isotopes are very useful to get information related to the nutritional assimilation (Seminoff *et al.*, 2006), even at great periods of starvation, as the breeding season. As suggested by Whiting *et al.*, (2007), the continental shelf constitutes an important foraging area for *L. olivacea*, with shallow feeding behaviors of surface and benthic prey. According to Hess *et al.*, (2008), the olive ridley sea turtle occupies the level 2, 3 and 4 of the trophic web, making them polytrophic consumers.

The aim of this study is to evaluate the combined use of morphometric, behavioral data, and biochemical indices (stable isotopes and biochemical indices based on nucleic acid derived indices) as predictors of the body condition, potential survival and reproductive

ability of the females and their hatchlings during their nesting season. This study may contribute towards the development of minimally invasive potential growth, the success of produce progeny, and population status measurement strategies, which are essential to gather more knowledge on this species, in order to plan future conservation strategies. Our specific aims were to investigate the following hypothesis, regarding adult females' sea turtles: (F1) Does the females' fitness decreases along the season?; (F2) Is the female size related with their physiological condition?, (F3) Does the *L. olivacea* show an oceanic feeding behavior? (F4) Is it useful to use biochemical indicators, as sRD and stable isotopes, as indicators of body condition of *L. olivacea*? and regarding the hatchling sea turtles: (H1) Is the hatchling condition dependent of females condition?; (H2) Does the incubation temperature and time influences the reproductive success and locomotor performance of hatchlings?, (H3) Are hatchlings produced by "large" females larger than the hatchlings produced by "small" females?

2. Material and Methods

2.1. Study area

The study was performed in the archipelago of São Tomé and Príncipe, located in the Gulf of Guinea on the west coast of Africa ($0^{\circ}20'54.24''\text{N}$ $6^{\circ}44'11.40''\text{E}$), at 200 miles offshore Gabon. São Tomé is the main and largest island of the archipelago (Fig. 1).

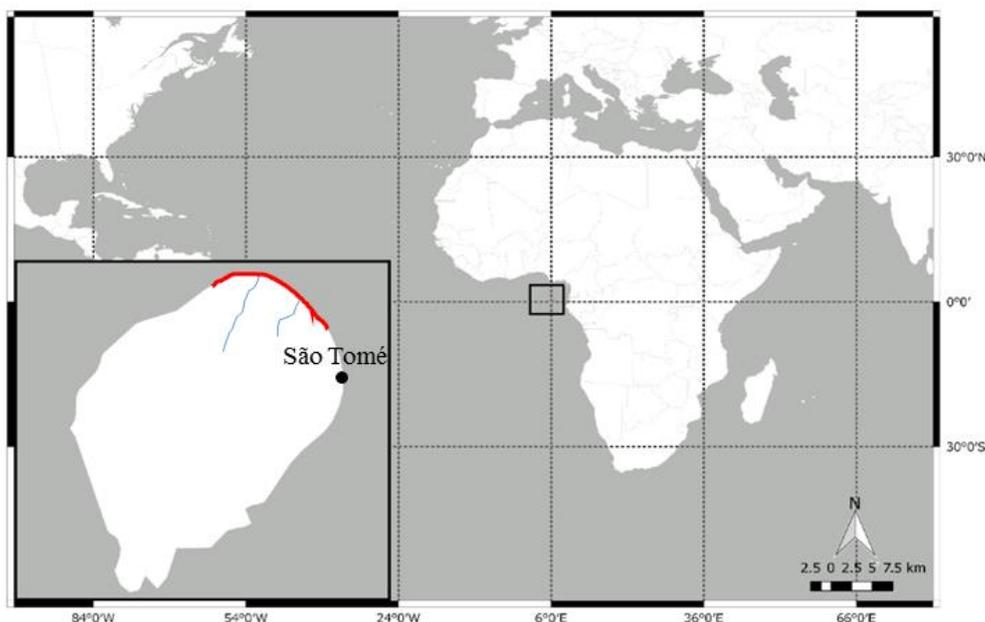


Figure 1. Map of São Tomé (São Tomé and Príncipe) and sampling area (red line).

São Tomé and Príncipe is an extremely poor country that has a great tradition of sea turtle harvesting, either in coastal waters or on nesting beaches. For many years, the protection and conservation of sea turtles in the archipelago were highly conditioned by the absence

of a law that could ban or punish these activities. São Tomé and Príncipe became signatory to CITES (Convention on International Trade in Endangered Species) in 2001. However, without an adequate legal framework, mortality rates ranged from 67 - 89% of nesting females each year. Intense campaigning and negotiation with São Tomé and Príncipe government for nearly a decade finally gave rise to the first national legislation for the full protection of sea turtles in June 2014. Nonetheless, the conservation of sea turtles in the archipelago is still a delicate issue.

L. olivacea is the most abundant sea turtle species in São Tomé Island. They nest every year between October to early April, with a nesting peak from late November to early February, mainly on the northern coast. The study was conducted on the main nesting area of olive ridley sea turtle on the archipelago, between the fishing villages of Micoló and Morro Peixe, north of São Tomé Island (ca. 10 km of beach).

2.2. Field work

The fieldwork was performed between November of 2015 and April of 2016. A total of 435 individual female and hatchlings sea turtles were measured throughout the study. Among these individuals, 107 females were sampled, in which 33 samples were also taken into account for the isotopic signal and sampled 328 random hatchlings from 57 selected nests.

Measurements and sample collection nesting females

The sampling of females was from 12th of November to 15th of February and the procedure consisted in night patrols and monitoring through the beaches. All the procedures started when the females were covering the nests, in which they do not respond to human contact (Perrault *et al.*, 2012).

Two small skin tissue samples were taken from the front flippers (<2 mg), using a 4-mm blade. The samples were preserved in two solutions, RNAlater at low temperatures and 100% alcohol diluted in ultrapure water (alcohol 70%) at room temperature. Nesting females were also measured (curved carapace length = CCL_{min}) and CCW (curve carapace weight= CCW_{min}) with a fiberglass tape measure. The females were also tagged in both front flippers. All the steps were executed using red lights in order to minimize the stress caused.

After the female return to the ocean, the nests were translocated to the hatcheries in Micoló and Morro Peixe. In the hatchery, artificial nests were reburied in the sand with the same characteristics/measurements as the natural ones. When the hatchery was full, some of the nests were maintained in their original location or translocated to another area of the beach, with more protection and under constant supervision.

Measurements and sample collection from hatchlings

The sampling of hatchlings was performed in 5 randomly selected individuals from each hatched nest, from 4th of January to 11th of April. During the season, 50 natural and artificial nests were excluded from the study because they could not be located on the beach or due to dog and/or pig predation or because they were lost, respectively.

CCL_{min} and CCW_{min} were also measured with a caliper. Effort tests were also made as the self-righting ability - the time that the hatchling takes to turn from upside-down to the normal position (self-righting ability), and the time that they take to reach the water at a distance of 5 meters from the foreshore (crawling velocity). Both tests failed when the hatchlings exceeded a minimum record of 60 seconds (did not turn) and 10 minutes (did not move), respectively. To study the RNA:DNA ratio between female/hatchling, a small biopsy was performed in the back flipper of the hatchlings and conserved in RNAlater.

The natural nests were relocated to the hatchery and the hatchlings were released at the moment of emergence, with the exception of the 5 individuals from each nest, which were kept in a pot during the night and until the 5 p.m. of the next day to be sampled and released in the nightfall.

The day after the big emergence, the nests were exhumed in order to know the number of dead/live hatchlings, empty shells, and shells with and without an embryo.

2.3. Laboratory analysis

RNA:DNA ratio

The assessment of the body condition of *L. olivacea* was through the sRD index (standardized RNA/DNA ratio), where it was possible to determine the quantity of DNA and RNA, in milligrams, per tissue.

Caldarone *et al.*, (2001) and Esteves *et al.*, (2000) summarized the methods to quantify nucleic acids for fish larvae, already used in females and hatchlings of sea turtles (see Vieira, 2014).

The quantification of nucleic acids was performed during all the nesting season in females and respective hatchlings, in order to distinguish two different populations, which come to spawn in São Tomé at two different times; in the beginning and in the end of the season.

The tissue samples were initially homogenized in two different ways: mechanically and chemically. The next step was the measurement of the fluorescence-photometric, using gel red (GR), which acts as a specific nucleic acid fluorochrome dye, and a microplate reader (Biotek synergy HT model SIAFRTD). It used an excitation wavelength of 365 nm and an emission wavelength of 590 nm.

Before adding GR, the endogenous fluorescence was also measured regarding the first group of samples for each tissue. It was negligible and discarded from the calculations for the concentration of nucleic acids.

The samples were cooled to room temperatures before reading the total fluorescence, and then they were incubated with ribonuclease A (Type-II A) at 37°C, during 30 minutes, for a second measurement. The fluorescence regarding the total RNA, mostly ribosomal, was calculated with the difference between the total fluorescence (RNA and DNA) and the second fluorescence, after receiving a ribonuclease treatment which is assumed to be due to DNA.

To determinate the concentrations of DNA and RNA, standard curves of DNA-EB plus RNA-EB were ran every day with known concentrations of μ -phagus DNA (0.25 $\mu\text{g } \mu\text{L}^{-1}$) and 10-23 s E.coli RNA (4 $\mu\text{g } \mu\text{L}^{-1}$), in an appropriate range if values.

The average ratio of DNA and RNA slopes was $5.76 \pm 1.4 \text{ SE}$.

After the average ratio of DNA and RNA slopes were known, the nutritional condition could be assessed, through nucleic acid derived indexes as sRD, DNA/mg Dry weight, and RNA/mg Dry weight. According to Caldaroni *et al.*, (2006), the RNA:DNA ratios were then standardized (sRD) using the average ratio of DNA and RNA slopes and the referenced slope of 2,4, defined by himself. It was used an excitation wavelength of 365nm and an emission wavelength of 590 nm for the fluorescence measurement.

Stable isotopes

Approximately 1.0 mg of skin tissue samples of olive ridley sea turtles were dried and smashed to homogeneous powder with a ball mill at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), in the University of Porto. The samples were then placed into sterilized tin capsules and analyzed by a continuous-flow isotope ratio mass spectrometer: Thermo Scientific Delta V Advantage IRMS via ConFlo IV interface (Marinova). The analytic error for both isotopic ratios was $\pm 0.01 \text{ ppm}$.

2.4. Data treatment

. The reproductive and emergence success were estimated through the following formulas:

$$\text{Eclosion success: } \frac{\# \text{ hatched eggs}}{\# \text{ total eggs (hatched eggs + unhatched eggs + pipped + live pipped)}} \times 100$$

$$\text{Emergence success: } \frac{\# \text{ hatched eggs} - (\# \text{ live hatchlings in nest} + \# \text{ dead hatchlings})}{\# \text{ total eggs (hatched eggs + unhatched eggs + ded pipped + live pipped)}} \times 100$$

All graphics and statistical treatment were carried out through the open source software R version 3.3.1 (R Development Core Team; www.r-project.org). The analysis of the data used linear models after the data distribution checked. Furthermore, the influence of independent (environmental/behavior) factors (earlier/latest of breeding season, great/small size of female) and dependent variables (self-righting ability, crawling speed to reach the sea) on dependent variables (ecophysiological condition of hatchlings and adult females based on sRD ratio and hatchling success) were analyzed. For all the cases we used 95% confidence interval ($p < 0.05$).

All the assumptions from the linear model were accepted, and if not the case, the data was transformed through the logarithmization of the variable “self-righting time” and arcseno square root for the percentages variables “eclosion rates” and “emergence rates”. The correlation between demographic, morphometric, locomotor performance and biochemical parameters were analyzed using 95% confidence interval ($p = 0.05$).

3. Results

3.1. Morphometry and demography

Average CCL or nesting females was 69.07 cm and average maximum, within a range of 64.50 to 74.50 cm. For the hatchlings, it was $CCL_{min} = 4.14$ cm, with a range from 3.75 to 4.44 cm.

The analysis of length of the females in respect to the length of hatchlings revealed that the size of the females was not correlated with the size of hatchlings (p -value: 0.3185; Table I). However, there is a tendency for hatchlings produced by “large” females to be larger than the hatchlings produced by “small” females (Fig. 2).

Hatchling success ranged from around 20% to 97% ($n=278$, $x=58.97$, $sd=21.22$, $me=58.73$) and emergence success ranged from around 21% to 97% ($n=278$, $x=69.00$, $sd=19.30$, $me=77.14$).

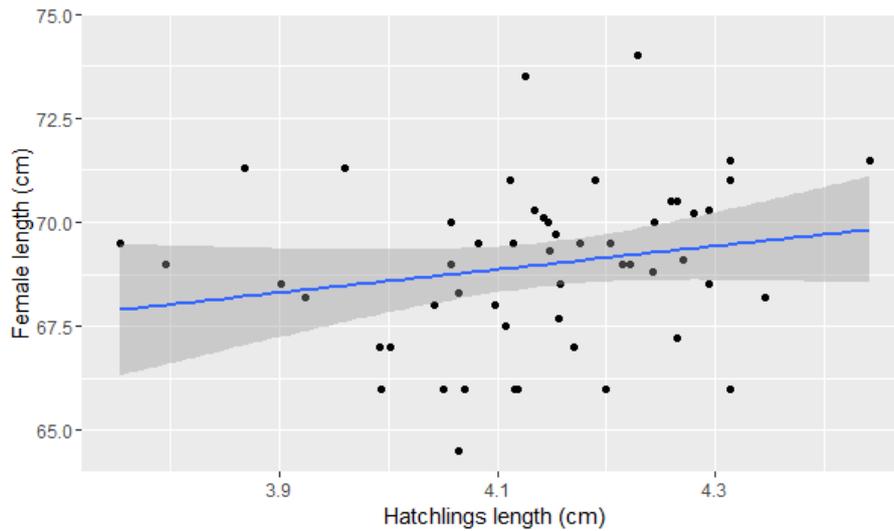


Figure 2. Relationship between the size of females and the size of hatchlings (p-value: 0.3959).

Table I - Results of one way-ANOVA tests for morphometry and demography. Legend: SS – sum of squares; DF – degrees of freedom; F-stat – F statistic; p – P-value. Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘ ’ 0.1 ‘ ’ 1.

ANOVA test	SS	DF	F-stat	p-value
Female length ~ Hatchling length	0.00859	1	0.7246	0.3959
Residuals	1.87372	158		
Incubation time ~ Eclosion success	504.9	1	29.974	9.982e-8 ***
Residuals	4564.6	271		
Temperature ~ Eclosion success	4883	1	11.76	0.0006994 ***
Residuals	112520	271		
Emergence ~ Eclosion success	72079	1	377.88	2.2e-16 ***
Residuals	52646	276		
Incubation time ~ Crawling speed	328.6	1	19.261	1.615e-05 ***
Residuals	4793.3	281		
Incubation time ~ Self-righting ability	71.69	1	17.438	3.975e-05 ***
Residuals	1142.89	278		
Temperature ~ Crawling speed	0.003513	1	11.955	0.0006311 ***
Residuals	0.081113	276		
Temperature ~ Incubation time	328.6	1	19.261	1.615e-05 ***
Residuals	4793.3	281		
Female length ~ Eclosion success	19.1	1	0.039	0.8443
Residuals	21991.5	45		
Hatchlings length ~ Eclosion success	458.9	1	1.7211	0.1957
Residuals	13063.7	49		

In respect to demographic parameters, the incubation time was positively correlated with the incubation temperature (p-value < 0.0001; Table I) (Fig. 3.A) and with eclosion success (p-value < 0.0001; Table I) (Fig. 4.A) that in turn is significantly positive for the incubation temperature (p-value < 0.0001; Table I) (Fig. 4.B). Nests at higher temperatures have lower incubation periods and have lower eclosion success (also known as hatchling success or reproductive success). Furthermore, the incubation time has

influence in both self-righting ability (p-value < 0.0001; Table I) (Fig. 5.B) and crawling speed (p-value < 0.0001; Table I) (Fig. 5.A). Overall, ANOVA test showed that hatchlings are faster when incubated at periods between 55 and 60 days. Hatchlings with incubation times lower than 55 days, seemed to have lower crawling speed. For the self-righting ability, the results showed that there was a clear pattern between incubation time and self-righting ability. Hatchling presenting an incubation time between 50 and 60 days had quicker self-righting ability.

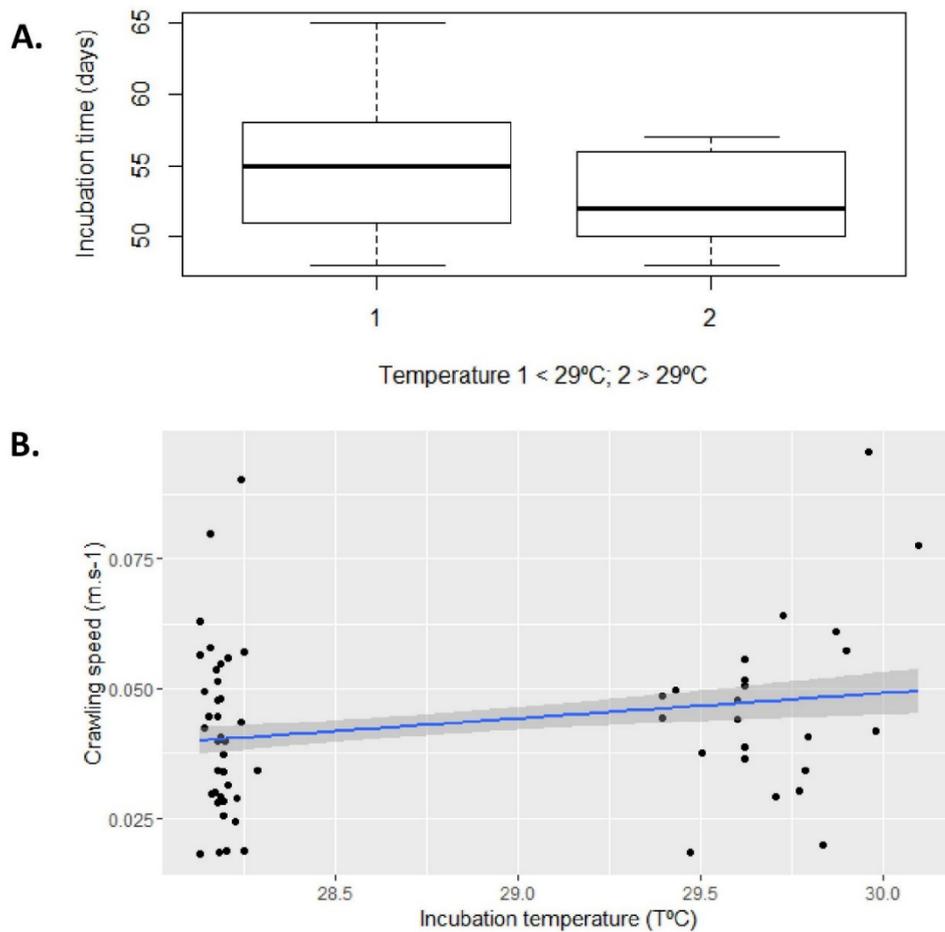


Figure 3. Incubation temperature. **(A)** Incubation time of eggs for two ranges of temperature (< 29°C and > 29°C) (p-value < 0.0001). **(B)** Relationship between the crawling speed of hatchling turtles and the incubation temperature of eggs (p-value < 0.0001).

The outcomes for the incubation temperature have shown that there is a correlation between the incubation temperature and the crawling speed (p-value < 0.0001; Table I) (3.B).

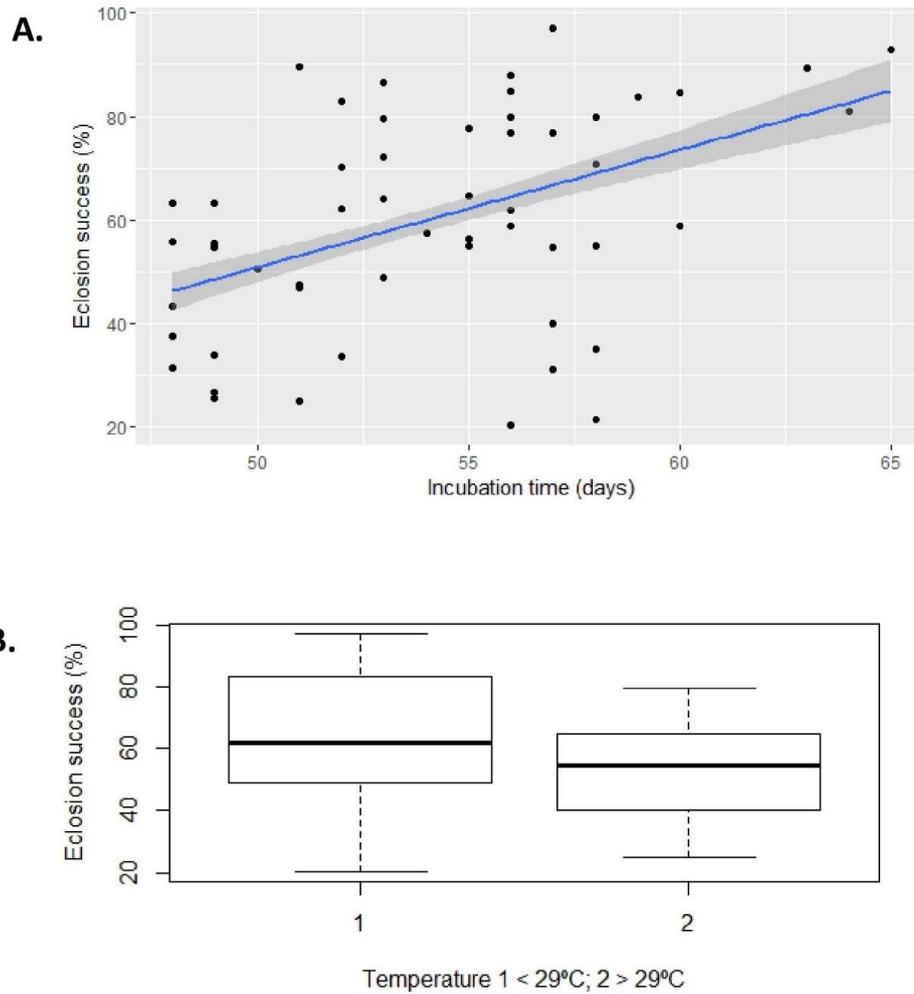


Figure 4. Eclosion success. **(A)** Relationship between eclosion success of the hatchling turtles and the incubation time of eggs (p-value < 0.0001). **(B)** Eclosion success rates for two ranges of incubation temperature of eggs (< 29°C and > 29°C) (p-value < 0.0001).

A positive relation between emergence and eclosion success (p-value < 0.0001; Table I) was observed (Fig. 6), meaning that hatched eggs have high emergence rates.

Neither the size of females or hatchlings revealed to have influence in the eclosion success (p-value: 0.8443 and p-value: 0.1957, respectively; Table I) (Fig. 7.A and Fig. 7.B).

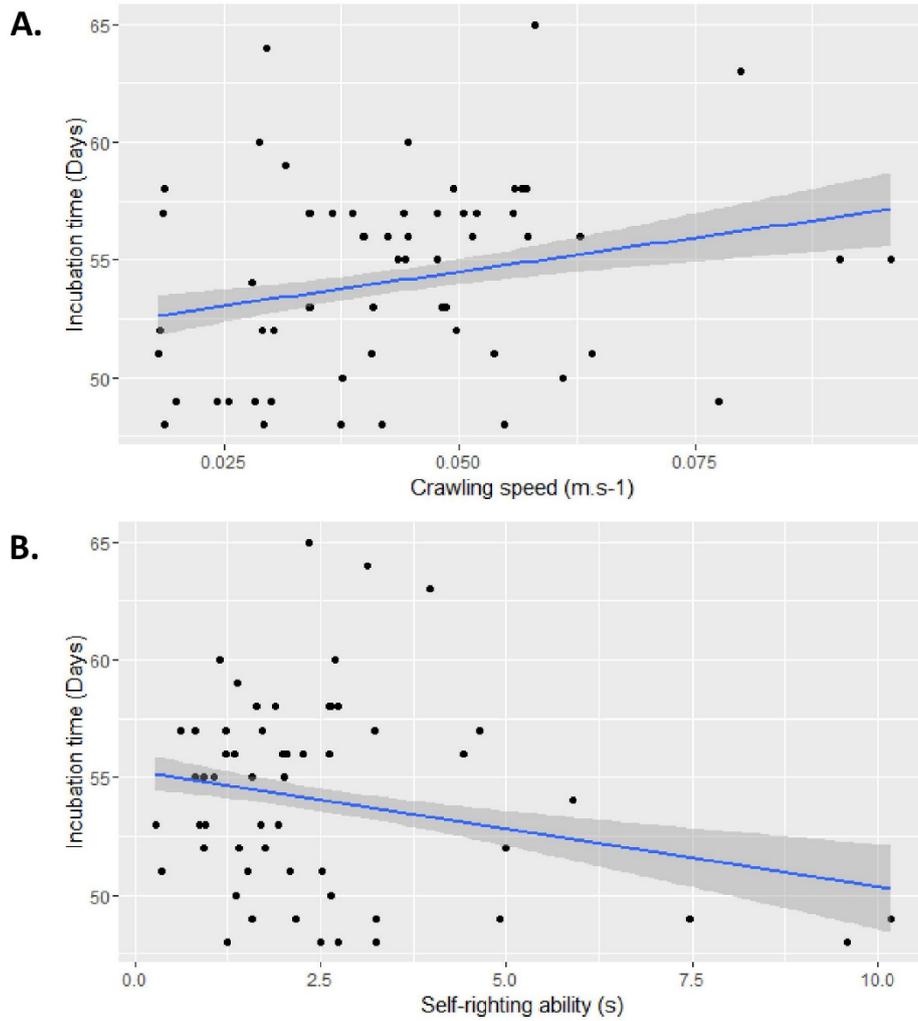


Figure 5. Incubation time of eggs and locomotor performance of hatchling turtles. **(A)** Relationship between the incubation time of eggs and the crawling speed of hatchling turtles to reach the sea (p-value < 0.0001). **(B)** Relationship between the incubation time of eggs and the self-righting ability of hatchling turtles to turn upside down (p-value < 0.0001).

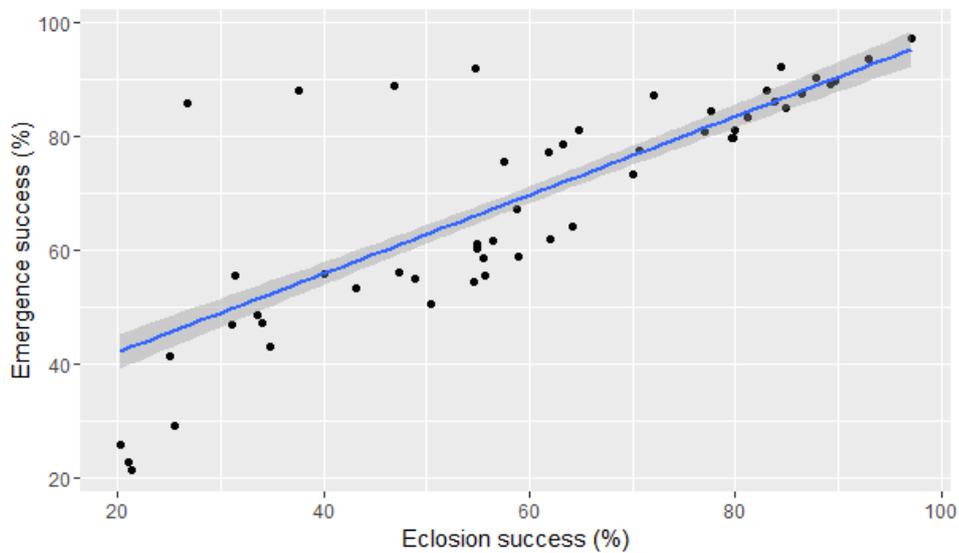


Figure 6. Relationship between the emergence success and the eclosion success of hatchling turtles (p-value < 0.0001).

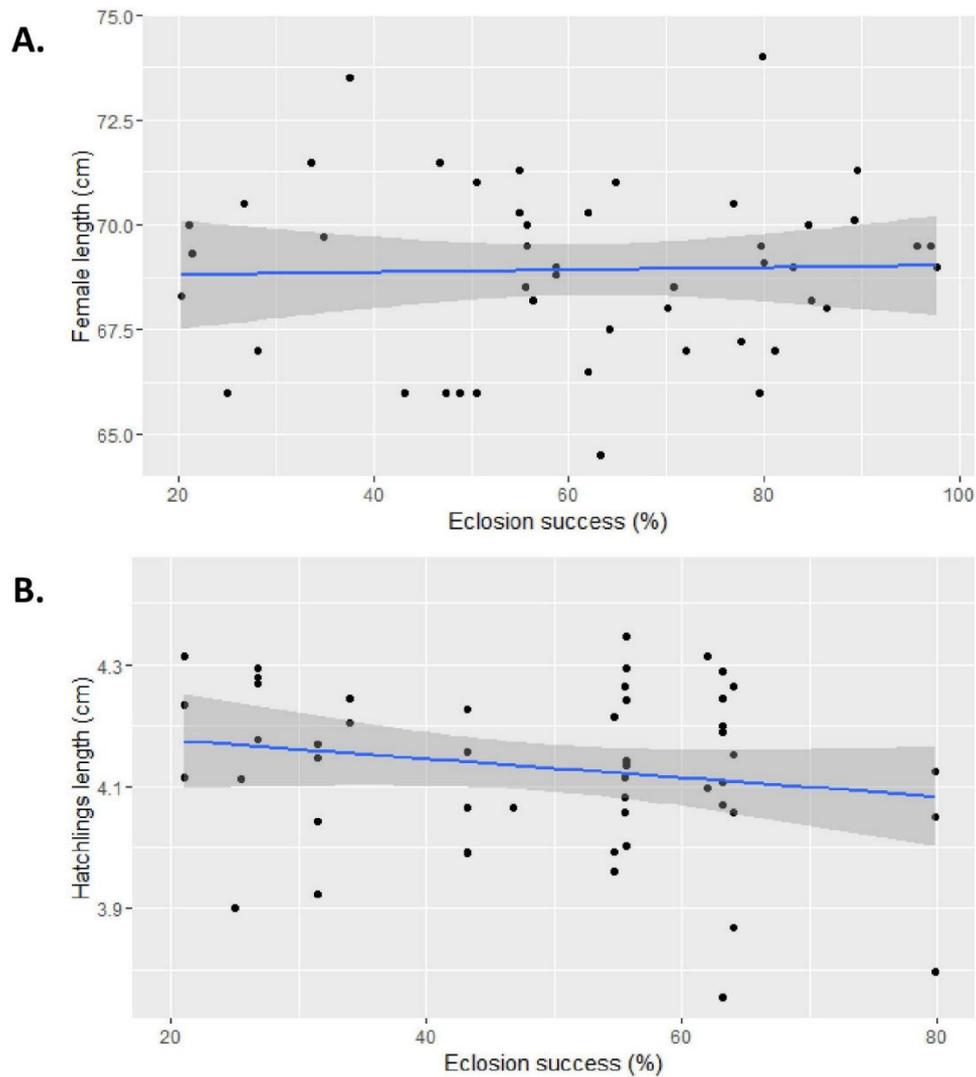


Figure 7. Females and hatchling turtles' length and the eclosion success. **(A)** Relationship between the size of the females and the eclosion success of hatchling turtles (p-value: 0.8443) **(B)** Relationship between the size of the hatchlings and the eclosion success of them (p-value: 0.1957).

3.2 Nesting females: biochemical indices

The ecophysiological condition, of nesting females, measured through the sRD was not significant among seasons (p-value: 0.8241; Table II) (Fig. 8.A), however, it is possible to observe a trend for females in the latest season have higher values of sRD. The sRD values were also not affected by turtle size (p-value: 0.3029; Table II) (Fig. 8.B).

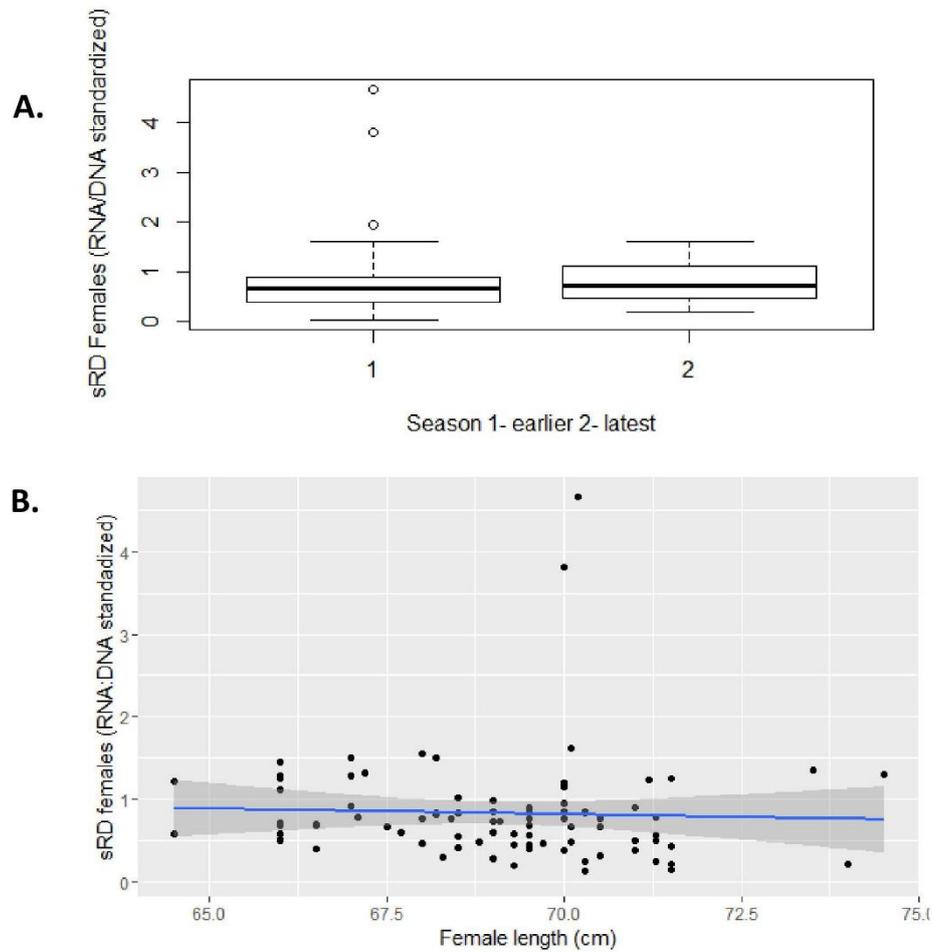


Figure 8. Biochemical condition of females and season/length. **(A)** Standardized RNA/DNA ratio for nesting females in the beginning of the season (Earlier) (11th of November of 2015 to 12th of December of 2015) and at the end of the season (Latest) (14th of December of 2015 to 11th of February of 2016) (p-value: 0.8241). **(B)** Relationship between the physiological condition (sRD) of females and their size (p-value: 0.3029).

Table II - Results of one way-ANOVA tests for biochemical indices of nesting females. Legend: SS – sum of squares; DF – degrees of freedom; F-stat – F statistic; p – P-value. Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘.’ 1.

ANOVA	SS	DF	F-stat	p-value
sRD female ~ Season	0.019	1	0.0496	0.8241
Residuals	40.326	104		
sRD female ~ Female length	0.01398	1	1.0743	0.3029
Residuals	1.10579	85		
$\delta^{13}\text{C}$ ~ Female length	4.3805	1	8.154	0.008333 **
Residuals	13.9676	26		
$\delta^{15}\text{N}$ ~ Female length	27.208	1	6.1982	0.01951 *
Residuals	114.133	26		
sRD female ~ Eclosion success	60	1	0.1213	0.7292
Residuals	23240	47		
sRD female ~ Emergence	261.5	1	0.6174	0.436
Residuals	19905.8	47		

In general, the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ showed two groups of organisms in terms of their isotopic composition content. A major group represented by organisms with higher carbon and nitrogen content, and a small group of turtles with low carbon and nitrogen content (Fig. 9.A) (Table II).

We measured the isotopic composition of nesting females of *L. olivacea* in adult stages and correlated it with their size. The analysis showed that CCL had an effect on the content in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ it (p-value < 0.001 and 0.01, respectively; Table II) (Fig. 9.B and fig. 9.C).

The outcomes of the physiological condition of females in respect to eclosion and emergence success did not reveal any correlation (p-value: 0.7292 and p-value: 0.436, respectively; Table II) (Fig. 10.A and fig. 10.B).

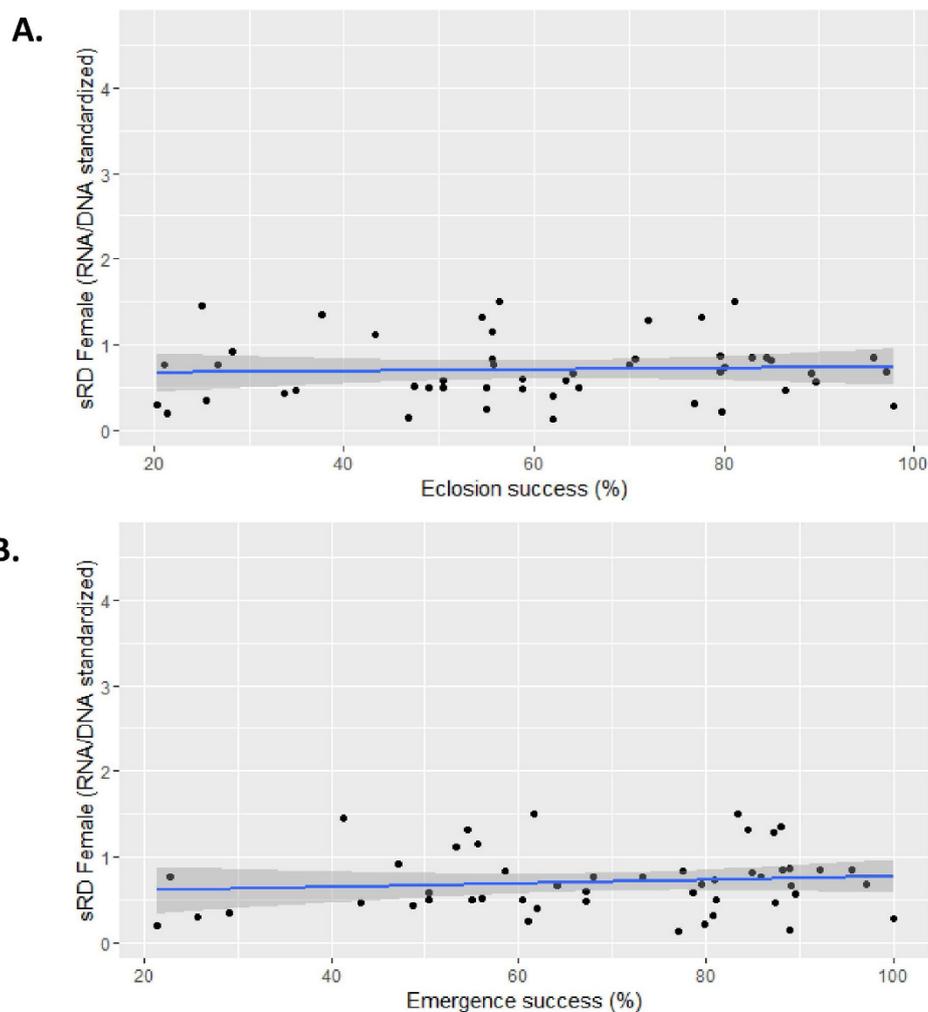


Figure 10. Biochemical condition of females and reproductive success. (A) Relationship between standardized RNA/DNA ratio and the eclosion success of hatchling turtles (p-value: 0.7292) (B) Relationship between standardized RNA/DNA ratio and the emergence success of hatchling turtles (p-value: 0.436).

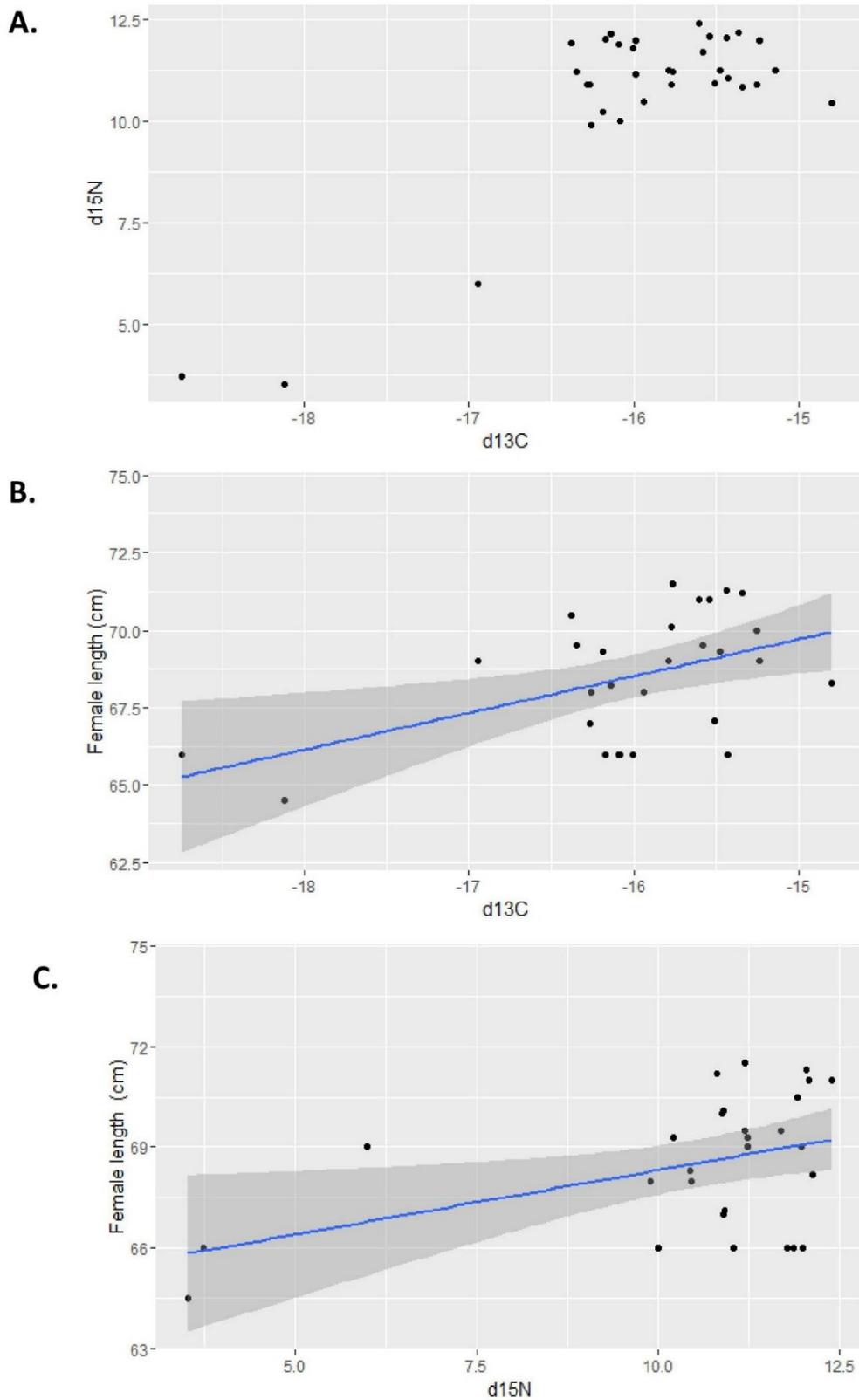


Figure 9. Isotopic composition. **(A)** Relationship between carbon and nitrogen content of nesting females. **(B)** Relationship between the size of the females and their content in carbon-13 (p-value < 0.001). **(C)** Relationship between the size of the females and their content in nitrogen-15 (p-value < 0.01).

3.3. Hatchlings turtles: Biochemical and behavioral indices

The ecophysiological condition of the hatchlings was not positively correlated with the ecophysiological condition of the females (p-value: 0.3959; Table III) (Fig. 11.A). However, the physiological condition trend for hatchlings is slightly higher than for the females. Plus, the condition of the hatchlings did not show to be positively correlated with their size (p-value: 0.6208; Table III) (Fig. 11.B)

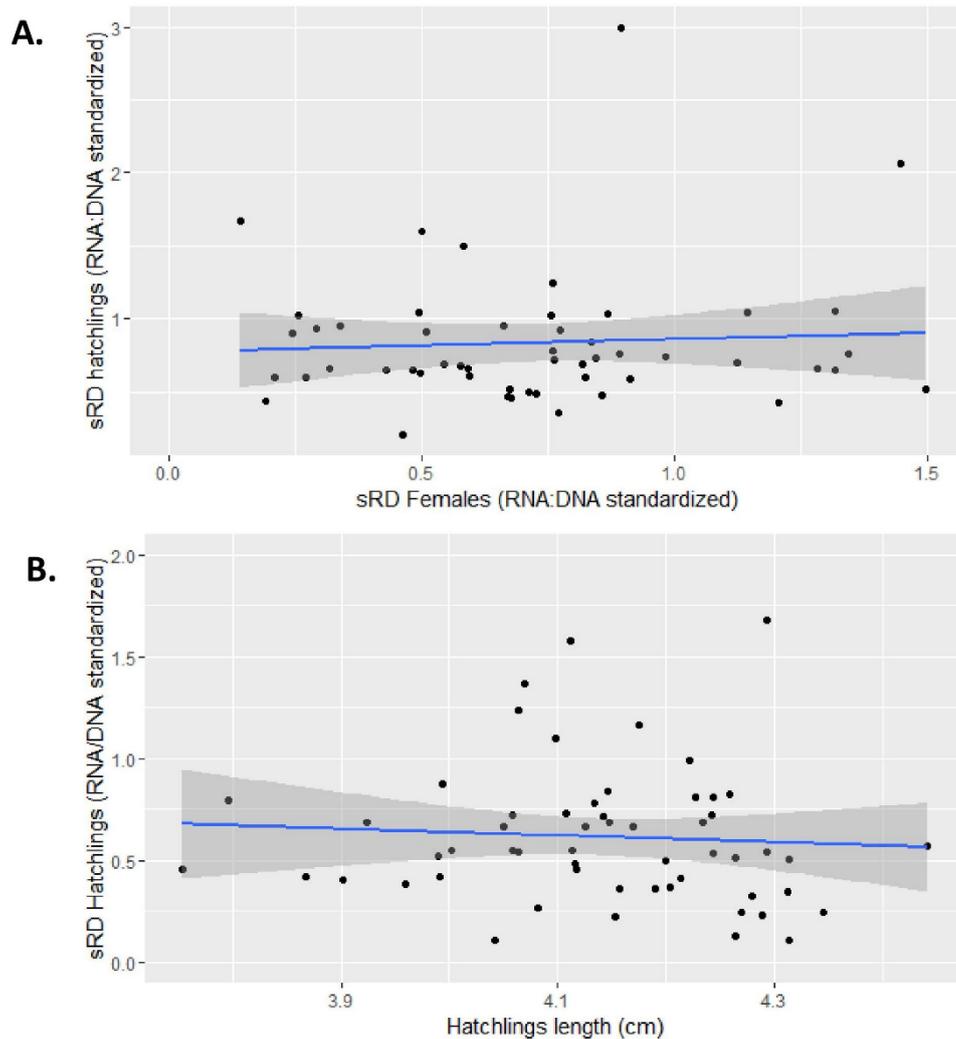


Figure 11. Biochemical condition of hatchling turtles. **(A)** Relationship between standardized RNA/DNA ratio of hatchlings and females (p-value: 0.833). **(B)** Relationship between standardized RNA/DNA ratio of hatchlings and their size (p-value: 0.6208).

Table III - Results of one way-ANOVA tests for biochemical and behavioral parameters of hatchling turtles. Legend: SS – sum of squares; DF – degrees of freedom; F-stat – F statistic; p – P-value. Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘.’ 1.

ANOVA	SS	DF	F-stat	p-value
sRD hatchlings ~ sRD females	0.0057	1	0.0449	0.833
Residuals	6.5048	51		
sRD hatchlings ~ Hatchlings length	0.00487	1	0.2476	0.6208
Residuals	1.06268	54		
Hatchlings length ~ Crawling speed	0.12022	1	6.7312	0.01228 *
Residuals	0.92872	52		
Hatchlings length ~ Self-righting ability	0.075	1	0.1572	0.692
Residuals	153.409	321		

Although the size of hatchlings did not reveal any significant difference regarding the self-righting ability (p-value: 0.8527; Table III) (Fig. 12.B), the crawling speed was influenced by their size (p-value < 0.01; Table III), meaning that “large” hatchlings were also faster (Fig. 12.A).

A summary of the results for the established hypothesis in our aims is presented in table IV.

Table IV - Summary of results for the hypothesis in the present study.

Hypothesis	Validation
F1 - Does the females’ fitness decreases along the season?	Not accepted
F2 - Is the female size related with their physiological condition?	Not accepted
F3 - Does the <i>L.olivacea</i> show an oceanic feeding behavior?	Accepted
F4 - Is it useful to use biochemical indicators, as sRD and stable isotopes, as indicators of body condition of <i>L. olivacea</i> ?	Accepted
H1 - Is the hatchling condition dependent of females condition?	Not accepted
H2.A - Does the incubation temperature and time influences the reproductive success of hatchlings?	Accepted
H2.B - Does the incubation temperature and time influences the locomotor performance of hatchlings?	Accepted
H3 - Are hatchlings produced by “large” females larger than the hatchlings produced by “small” females?	Not accepted

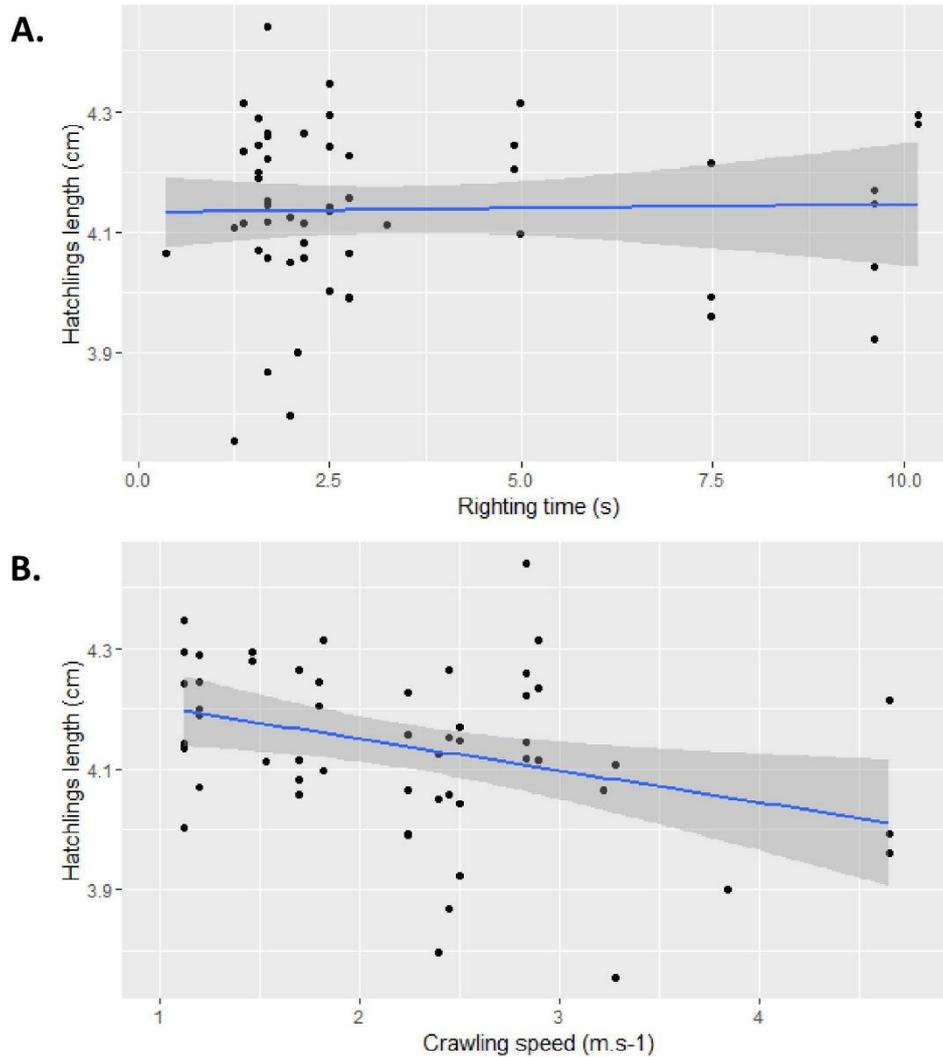


Figure 12. Hatchling turtles size and behavioral parameters. **(A)** Relationship between the size of hatchlings and their self-righting ability (p-value: 0.692). **(B)** Relationship between the size of hatchlings and the crawling speed (p-value < 0.01).

4. Discussion

The purpose of this study was to evaluate the body condition of *L. olivacea* using different fitness indicators to assess the individual health and population status. The major finding of this study was the independence of hatchlings condition from female ecophysiological status for the studied species (H1; table IV). This may be explained by a good condition of all females sampled, or by a great investment from females, that even with reduce ecophysiological status, may be able, at a high energetic cost to produce a progeny of high quality. This may be interpreted as an ultimate effort to contribute to the survival of their hatchlings. Although different strategies had been reported for sea turtles, e.g. by Vieira *et al.*, (2014) for *Caretta caretta* in Cape Verde, or by Areosa (2014) for *Chelonia mydas* in Principe Island this may be a site or specie specificity.

4.1. Nesting females

Life history traits determine the schedule and duration of key events in an organism lifetime which are shaped by natural selection to produce the largest possible number of surviving offspring. The variability of condition and size of females and hatchling registered in this study may be related to one aspect of natural selection that we need to pay more attention, to bet-hedging that can be summarized as a way to diversifying maternal investments because over the long term we know that environments are quite variable. Bet-hedging allows the turtles to survive despite those fluctuations as it reduces the chance all out failure. This strategy allows for a diversity of genotypes that includes traits that may not be advantageous in the short term, but will ensure that an organism survives in a particular extreme environment. In an unpredictable environment, one expects bet-hedging to evolve (Sæther and Engen, 2015; Morita and Nakashima, 2015; Olofsson *et al.*, 2009; Crean and Marshall, 2009; De Jong *et al.*, 2011)

In general, sea turtles store a high amount of energy while in foraging areas for several years that will be used during the reproduction season. The females return to nesting beaches in the same region they hatched (as known as ‘philopatry’) and it is believed that the interval they need to return to these beaches varies according to the quantity and quality of food available in the foraging grounds (Limpus, 1993; Miller, 1997). During the migration period and nesting season, females pass through long periods of starvation while nesting on an average of two times (Plotkin *et al.*, 1995; Tripathy and Pandav, 2007; Whiting *et al.*, 2007b), each year (Da Silva *et al.*, 2007). Changes in the food uptake during the nesting season is expected to influence biochemical indicators RNA/DNA ratios, and especially RNA concentration. Several studies (Perrault *et al.*, 2014; Goldberg *et al.*, 2013; Hamman *et al.*, 2002) performed in other species of marine turtles reported that along the nesting season the biochemical condition should decrease, because they do not feed and are concentrated only in the nesting process, that requires high levels of energy expenditure.

Additionally, Meyer *et al.*, (2012) observed for several species of marine fishes that the biochemical condition decreased exponentially under starvation periods. However, in contrast to those findings, our results showed that nesting females did not exhibit a lower biochemical condition at the end of the season, but the opposite, even not significantly positive (F1; table IV). According to Hamman *et al.* (2003), this finding may be related with the presence of two different sub-populations that nest in São Tomé; one in the beginning of the season and the second at the end of the season. A possible explanation can be related to the distance that separates different foraging grounds from the nesting area (Areosa, 2013). Therefore, females that migrate from remote places arrive late in the breeding zones.

Meyer *et al.* (2012) observed that starvation signal can be expressed in muscle tissues, even though it is only a small part of the physiological response to resource limitation. Although the biochemical condition of females was not correlated with their size, this study have shown degrees of healthiness for different individual sizes of females (F2; table IV), meaning that small females have higher values of sRD, possibly because they have better foraging strategies, thus accumulating more energy or because between the mating and migration period they are more efficient and faster than the “large” ones. Likewise, in the nesting season, “small” females are clearly more effective and profitable. They are faster when coming up and going down in the sand and in the nesting process, conserving more energy and reserves along the season. Therefore, the “small” females are likely to have a higher growth potential (Chícharo and Chícharo (2008) and Areosa (2013). Roark *et al.* (2009) also stated that turtles have a favorable growth if they have better food reserves, compared to the individuals passing through starvation periods.

Growth rates and functions in sea turtles as in other animals are influenced by resource availability and quality as well as the conditions of the surrounding environment (Dunham, 1978). Defining trophic levels and foraging grounds and resources of endangered species is important for a better understanding of the different areas they choose and for the establishment of better conservation measures, once they rely on the different habitats for individuals at different stages of life. The use of stable isotopes in females of sea turtles is very useful because provides information regarding their foraging activity during the nesting season. Studies related to the stable isotopic ratio has shown to be a very powerful tool to study the diet composition and feeding behavior of many vertebrates, mainly in those species in which the traditional methods are not easy to apply (Rubenstein & Hobson (2004). Most of the studies related to the feeding habits of this specie revealed that *L. olivacea* is carnivorous, mainly in immature stages, but they can also be omnivores and scavengers in adult stages (Behera *et al.*, 2015) with an unspecialized diet (Varo-Cruz *et al.*, 2011). In the marine realm, $\delta^{13}\text{C}$ indicates inshore versus offshore feeding habitat (Hobson 1993; Hobson *et al.*, 1994) or pelagic versus benthic contribution to food intake (Dunton *et al.*, 1989; Hobson *et al.*, 1994; France, 1995; Burton and Koch, 1999; Jennings *et al.*, 1997) and marine or freshwater habitat use (Smith *et al.*, 1996), while $\delta^{15}\text{N}$ measurements serves as indicators of a consumer's trophic level (McCutchan *et al.*, 2003; Kelly, 2000), through food enrichment.

Although our range of isotopic values suggests a general similar feeding behavior, the gap in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for three individuals represent the division between two foraging groups. The three of them with significantly lower values of $\delta^{15}\text{N}$ and higher values of $\delta^{13}\text{C}$ can be smaller and feed in more detritic zones with freshwater close to the continental shelf, suggesting a small contribution from neritic preys in their diet (McClellan *et al.*, 2010). This indicates that coastal zones can be important foraging habitats for this specie (Whiting, 2007), although it has been showed by our results and

previous studies that they have a more remarkable oceanic feeding behavior (Luschi *et al.*, 2003). Such finding corroborates with Polovina (2003), that reported that olive ridley sea turtles dive deeper in oceanic realms with deep scattering layers, justifying their oceanic habitat and feeding behavior (F3; table IV). However, according to Caldwell *et al.*, (1969), they can also be bottom feeders once mud and sand were encountered mud and sand in their stomach.

The second group of nesting females indicates that the isotopic signal could be influenced by external abiotic factors such as weather. In São Tomé, the equatorial weather provides a lot of precipitation with strong runoffs from the land to the ocean, mainly in the river downstream. Moreover, the isotopic signal is greatly influenced by the amount of food availability. The turtles could be sampled during periods of higher runoff levels from the neighborhood's rivers and for this reason, the ocean water could be fresher or the sampled individuals were recent arrivals that had been foraging in different habitats before arriving São Tomé coast to nest.

Natural abundance of $\delta^{15}\text{N}$ (10.0 - 12.5) were registered in this study. It is possible to affirm that nesting females are not feeding during the nesting period and have a higher trophic level once they are feeding on organisms that already fed in others. Herewith, our results have demonstrated that the size of the females have influence over the $\delta^{15}\text{N}$ composition, meaning that small females are likely to have poor $\delta^{15}\text{N}$ signatures. It may happen because smaller individuals are feeding directly in more primary producers, accumulating less nitrogen, once they cannot digest complex or bigger food resources as fishes and invertebrates. This corroborates what Post (2002) stated in which the range of $\delta^{15}\text{N}$ values could be indicative that sea turtles are feeding over several trophic levels if the nitrogen signal at the food web base is stable for every foraging area. Nonetheless, if the nitrogen isotope value at the food web basin change with the foraging area, the differences in the isotopic signal will be more concrete of habitat than trophic level, because the isotopic signal of the preys will switch according to these areas. Thus, our results suggest that sea turtles have been feeding in different areas but the source of food is the same. As reported by Hobson (1993), the individuals with elevated abundances in $\delta^{15}\text{N}$ are more likely to have a poor nutrition due to starvation periods that cause the metabolism of the consumer to use their own protein tissues.

Concerning the $\delta^{13}\text{C}$ signature, its higher content suggests that female turtles are feeding in more benthonic areas, close to the land, while the others with lower levels of $\delta^{13}\text{C}$ are feeding in more pelagic zones, at higher trophic and salinity levels.

In order to have concrete results, a sampling of putative preys would be very useful to characterize the degree of interactions between predator and preys, to assess the specificity of prey items and to better understand their diet composition and foraging behavior.

4.2. Eggs and hatchling turtles

The accumulation of energy by the females before the nesting season is not only important for the individual fitness but also for the healthiness of the clutches (Hamman *et al.*, 2002). As we assumed that each female came to nest in São Tomé only once, the results regarding the sRD appear to be higher for hatchlings, thus not affecting their physiological condition.

As reported by Chicharo and Chicharo (2008) it is important to define basal levels as known as “critical ratio” in order to classify an organism as starving or healthy. However, this minimum ratio, which according to Buckley *et al.* (1984) correspond to the value when the protein growth, is null for a certain temperature. In this study was not possible to determine the protein growth because we could not create artificial stress conditions to the studied turtles. All were studied and maintained in conditions the closest possible to nature (see methods section). Nevertheless, our results remain reliable because the sRD was compared within individuals and according to different situations as seasons, female physiological condition, and size.

Another possible explanation for our results associates the moment of hatching eclosion with the moment of releasing them to open sea. Once the nests were maintained in the hatchery, the sampled hatchlings were only released to the ocean in the day after. In the meanwhile, they were maintained in a dark and cool place, yet a natural energy expenditure was unavoidable during that period. Furthermore, olive ridley sea turtles usually select beaches with high levels of humidity, near to river mouths or estuaries (Lopez-Castro *et al.*, 2004). Although it is possible to corroborate this finding with our results, once most of the females chose areas next to the small rivers, the fact that the nests were moved to the hatchery, with different characteristics, could have been influenced hatchlings condition.

Once our nests were relocated to the hatchery, it was not possible to verify if this is a determinant factor in the hatchlings survival and/or condition. However, the nesting beach selection may be a relevant factor in the reproductive success (Lopéz-Castro *et al.*, 2004). Thus, it is expected that nesting females will invest time to choose the most suitable location and time for nesting, within an area (Hamel *et al.*, 2008). Any type of nests' relocation is expected to have great effects on hatchling success (Limpus *et al.*, (1979); Mortimer (1999); Avella *et al.*, (2007); Wyneken *et al.*, (2007).

Demographic parameters, such as the incubation temperature and time, and reproductive and emergence success are supposed to have effects in the size and locomotor performance of turtle hatchlings (Packard and Packard, 1988). In respect to our results, the incubation temperature has shown to be a determinant factor in the time they took to reach the sea, indicating that hatchlings are faster when incubated at lower temperatures (H2.B; table IV). The explanation binds to the fact that at lower temperatures, hatchlings

take more time to develop and thus they retain every maternal reserve for their embryonic development making them more resilient. This is imperative for the safety of hatchlings, once they take less time to reach the sea and decrease their possibilities of desiccation due to overheating from sun exposure (Vieira *et al.*, 2014). Furthermore, to run away from the predators this ability is critical.

Another determinant factor in the survival of hatchlings in their way to the ocean for the first time is the crawling speed. Although it was not significantly correlated, our results have shown a trend for bigger hatchlings reach the water quicker than the small ones. The locomotor performance of sea turtles on land appears is difficult and as results, hatchlings may overturn or become trapped in marine debris (Davenport, 1997). It is important that hatchlings turtles have the ability of turn upside down themselves in this type of situations, to have a better chance of survival the journey until the ocean.

Sea turtles that have nutritionally enhanced development are larger and have better reproductive potential (Van Buskirk and Crowder, 1994). They lay larger eggs and consequently produce large hatchling turtles. Even though the size of the eggs was not studied, our results confirmed a tendency for “large” females to produce “large” hatchlings and the vice-versa for “small” females (H3; table IV). Eder *et al.*, (2012) also verified that although eggs are not correlated with the body size of nesting females, the size of hatchlings is, and this condition was also found in our results. The offspring size is, in fact, determinant in the fitness of many reptiles (Vieira *et al.*, 2014) and crucial to their survival chances. Once they are born, turtle hatchlings need to make their way to the ocean and the predation rates for sea turtles are higher in this period of their life because they need to cross the beach, trying to escape from diverse predators. Additionally, larger sizes may have some more advantages as faster swimming and to easily handle larger prey items (Ji *et al.*, 2002; Gyuris, 2000).

The developmental environment is crucial for oviparous species (Boleli and Queiroz, 2012) as it can affect the embryogenesis through the exposition of eggs to extreme environmental variations. Temperature is one of the most important factors because it affects the incubation period, the egg water loss during this period and subsequent has great influence in the embryonic viability and hatchlings condition (Brooks *et al.*, 1991; Booth 2000; Hewavisenthi and Parmenter, 2001; Reece *et al.*, 2002; Deeming, 2004, Georges *et al.*, 2005). The effects of the temperature are diverse and can be related with the sex determination (warmer temperatures produce more females while cooler temperatures produce more males) (Standora and Spotila 1985), body shape, color, size and amount of yolk which is converted in tissue along the embryonic development (Burgess *et al.*, 2006), locomotor performance (crawling faster or swimming longer), growth rate and behavior (Booth *et al.*, 2004). Changes in these parameters are expected to influence hatchlings fitness.

According to the results obtained in this study, the incubation temperature has shown to be one of the main influential criteria in hatchlings performance (H2.A; table IV) and this is especially relevant in the actual context of climatic changes. Fordham *et al.* (2007) has stated that the optimal incubation temperature is an adjustment between the maximization of reproductive success and hatchling size and minimizing the incubation period. Several studies (Booth *et al.*, 2004; Read *et al.*, 2013; Wood *et al.*, 2014), reported variations in hatchlings' fitness according to the incubation temperature. Validating this findings, our data suggests the same for locomotor parameters such as crawling speed and a possibly optimal performance when incubated at mid temperatures. Therefore, our results showed that the ideal incubation temperature in São Tomé was less than 29°C, in order to obtain the highest rates of eclosion success.

As Boleli *et al.* (2012) verified, our results have shown that incubation temperature has an influence on the period of incubation, becoming shorter at low temperatures. This indicates that incubation temperature is one of the main determinants of embryonic development in sea turtles. Our results did not show that at higher temperatures, the size of the hatchlings decrease in carapace dimensions. However, it is possible to verify that incubation temperature affects their locomotor performance, mainly the crawling speed. Considering the effects of temperature on egg development for other oviparous species, previous studies in loggerhead, green and olive ridley sea turtles (Booth, 2006; Booth *et al.*, 2004; Booth and Astill, 2001; Booth *et al.*, 2013; Hamman *et al.*, 2007; Ischer *et al.*, 2009; Maulany *et al.*, 2012; Read *et al.*, 2013; Wood *et al.*, 2014), have demonstrated that at higher temperatures, the size of the hatchlings decreases in carapace dimensions, affecting their locomotor performance and eventually growth rates (Glen *et al.*, 2003). Increasing sand temperatures associated with climate change may therefore negatively impact hatching success and locomotor abilities of hatchling, even making some areas completely unsuitable for hatchling production at least during parts of the year (Hawkes *et al.*, 2014). Changes in phenology of nesting seasons may mitigate some of the predicted negative impacts of higher incubation temperatures associated with climate change. The implication is that hatchlings produced from warmer nests might be more susceptible to predation while swimming away from the nesting beach (Gyuris, 1994). However, there is little information on long-term impacts of incubation temperatures on the traits or fitness of marine turtles. More long-term studies of individual turtles are needed to fully understand the potential impacts of climate change on fitness associated with incubation temperatures.

The determination of eclosion success is very important for the management of nesting beaches chosen by the sea turtles, once it indicates which ones are more suitable and also to understand the health of nesting populations (Miller, 1999). Although our results showed a great reproductive success, we cannot relate them to the characteristics of the spawning beaches because the nests were translocated to hatcheries with different

characteristics from the original beach. In order to have reliable results, we should maintain some nests on the beach and perform the same experiments there.

Our findings stated that eclosion rates are very similar to the emergence rates which contrasts to what some authors said (Miller, 1997; Hamman *et al*, 2002), that eclosion rates should be smaller than the emergence success. Our results suggests that it can be due to the hatchery conditions, which are more favorable (less humidity, controlled temperature, and coarser sand, less or almost no predation in most of the cases and enough space for hatchlings to emerge). Nevertheless, Hawles *et al*. (2014) suggested that some hatcheries location and characteristics, need to be re-think because usually host a higher density of nests and often further up the shore.

Contrary to the phenomenon (termed *arribada*) that takes place in few nesting beaches chosen by *L. olivacea*, mainly in Mexico (Kichler *et al.*, 1999; Fonseca *et al*, 2009; Ocana *et al*, 2012), India (Shanker *et al*, 2003; Tripathy and Pandav, 2007), Costa Rica (Jensen *et al* 2006; Fonseca *et al*, 2009), the solitary-nesting behavior that occurs in São Tomé and in other numerous beaches around the world, has proved to have benefits for olive ridley sea turtle (Dornfeld, 2015). Corroborating with Dornfeld (2015) solitary nesting beaches are cooler and have higher hatchling success (H2.A). Contrary to this, in *arribada* nesting beaches are negatively affected by the high nest density for the oxygen availability and the incubation temperatures, which are higher, decreasing the hatchling success once they reach lethal incubation temperatures. Additionally, the nest destruction by other nesting females is a determinant factor in the emergence of hatchlings, compared to the eclosion success.

For long-lived individuals as sea turtles, our results, supported by the bet-hedging strategy, suggests that low annual reproductive effort of the nesting females will reduce the effect of low, stochastic hatchlings survival on the reproductive success of individuals (Cunnington and Brooks, 1996). This justifies the fact the *L. olivacea* reproductive strategy and why they come back to the nesting beaches at intervals of two years and this will increase the survival chances despite the environmental fluctuations that nesting females can face. Even though, sea turtles do not need to fight against the unpredictable abiotic conditions, as fish larva that is more susceptible at hatching, to environmental variables (Chicharo *et al.*, 1998), it is important for them to have evolutionary strategies that will maximize the survival chances through changes in their physiology and behavior, in order to generate a well-adapted offspring. From an evolution point of view, the individuals' fitness is a central concept (Saether and Engen, 2015) and by this reason the hatchlings condition should be high, otherwise their fitness would be highly variable among years, without taking into account anthropogenic effects, as adults' fishing, eggs poaching, pollution and beach development (Hamel 2008).

Conclusion

Our major findings showed a diverse feeding strategy for *L. olivacea*, that rely both on benthonic and pelagic items, and a reduce variability in their ecophysiological condition. This may reflect an adequate nutritional status of the females sampled in the studied area, nevertheless, the higher variability of hatchling condition may anticipate different responses in terms of potential survival.

Our results also showed that larger hatchlings of *L. olivacea* are more actively physically (righting and crawling) than smaller ones, which means they have high survival chances once they can escape easily from different types of predators, swim faster and handle larger preys. Additionally, the temperature is the most sensitive studied parameter, having great influence in the reproductive success and locomotor performance of the offspring. Similarly, the incubation time is very sensitive and important for the locomotor performance of hatchlings, i.e., the more the incubation period the faster are the hatchlings. These results tied with incubation temperatures highlight the importance of our results in terms of present scenarios of climate changes. This may involve new management strategies related to hatcheries location and characteristics, which usually host a higher density of nests and often further up the shore.

We can also conclude that the combination of morphometric, behavioral measurements, and biochemical indices based on minimally invasive techniques, such as nucleic acids concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ concentrations, may offer us an adequate way to assess individual fitness and population health status of endangered species, as sea turtles, with an increased needed of been monitored to face climate change.

Available data suggests that although *L. olivacea* displays a different behavior to other Atlantic turtle species, their main purpose is still to maximize the fitness. This represents a trait that is preserved along species radiation and evolution.

Gaining information on fitness, growth rates, reproduction potential and health status of hatchlings is fundamental to implement adequate management strategies in the future. However, the utility and validation of these indices on *L. olivacea* deserves further research, especially the development of new ways of assessment of hatchlings vigor with minimum evasive manipulation, not only self-righting time or crawling speed but other behavioral tests with potential interest, e.g. initial swimming abilities, crucial to avoid first predation events.

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