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Physiological effects of incidental capture and seasonality on juvenile green sea turtles (*Chelonia mydas*)

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ABSTRACT

Fishery is one of the main threats faced by sea turtles. However, literature relating to the physiological effects of incidental capture by fishing gears is scarce. The aim of this study is to establish blood reference values for a juvenile population of *Chelonia mydas,* from Ubatuba- Brazil, and compare them with incidental captured animals by fisheries in the same area, considering seasonal variation. Animals were captured during summer and winter, from direct capture (diving) and incidental capture (pound net and gillnet). Blood samples were collected from 96 healthy green sea turtles and, were analyzed for determining levels of glucose, lactate, total protein, uric acid, albumin, total lipids, triglycerides, VLDL cholesterol, total cholesterol, corticosterone concentrations, total antioxidant capacity, and lipid peroxidation. Corticosterone concentrations were significantly lower in animals captured by diving than in those captured by pound net and gillnet and were thought tso more closely represent baseline levels. Thus, the values found in animals captured by diving were used to establish reference values for this population. Turtles captured in summer had a significant increase in blood levels of glucose, total protein, uric acid, albumin, TBARS, and TEAC; and a decrease in total lipids compared with turtles captured in winter. Although pound nets are considered a low impact fishery to the turtles due to the low mortality rates, seven out of twelve parameters differed significantly from baseline values established for the species. Entanglement in gillnets caused greater perturbations than pound net and all parameters analyzed were significantly different from the reference values. These data indicate that incidental capture causes substantial alterations in health parameters of sea turtles. The results obtained in this study would help in future rehabilitation programs of sea turtles that are captured by fisheries. Additionally, reference values can be used for future comparisons with populations of the same species and with unhealthy and stressed individuals.

1. Introduction

Sea turtles face many threats during their life cycle; however, incidental capture by fishing gears is one of the main factors of populations decline around the world (National Research Council, 1990; Wallace et al., 2010) and in the southeastern coast of Brazil (Tagliolatto et al., 2020). Fisheries interaction is a problem of increasing concern, because despite the turtles being frequently released alive, the ultimate fate of these animals is unknown (Gearhart, 2001). Severe disruptions in normal physiological functions, and injuries suffered while entangled in gillnets, could lead to undocumented deaths and an underestimation of sea turtle mortality (Harms et al., 2003; Stabenau and Vietti, 2003; Snoddy et al., 2009).

Snoddy and Williard (2010) used satellite telemetry to monitor post-release movements of sea turtles released from gillnets and estimated that the mortality of these animals could be from 7.1 to 28.6%. They also verified differences in plasma ions (K^+, Cl^- e Na^+) and lactate levels, comparing turtles that died (confirmed mortality) with other sea turtles released from gillnets.

It is known that during forced submergence, sea turtles show different physiological responses when compared to a normal dive (Stabenau et al., 1991; Lutcavage and Lutz, 1997). These differences occur mainly due to the aggressive behavior while trying to free themselves from gillnets, accelerating the metabolism and rapidly

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consuming oxygen reserves, resulting in a low tolerance time to anoxia (Lutcavage and Lutz, 1997).

The efforts to escape from gillnets and reach the surface to breathe also result in metabolic disorders. Sea turtles entangled in gillnets and shrimp trawls exhibited metabolic and respiratory acidosis with high levels of lactate, PCO_2 and PO_2, and changes in ions concentration (Na^+, K^+, Cl^-), as well as decreased blood pH and increased respiratory frequency (Stabenau et al., 1991; Hoopes et al., 2000; Harms et al., 2003; Stabenau and Vietti, 2003).

By contrast, loggerhead turtles (*Caretta caretta*) captured in longline gear and pound nets did not show greater perturbations in lactate, glucose and pH levels in the blood, what can be explained by the free access to the surface to breathe during the capture. Although these levels were low, they are still higher than the reference values established for this species (Harms et al., 2003; Williard et al., 2015).

Gregory et al. (1996) observed that entanglement of loggerhead turtles in fishing gear can induce a systemic stress response, resulting in elevated levels of corticosterone and glucose. Snoddy et al. (2009) verified that green sea turtles (*Chelonia mydas*) captured in gillnets had higher levels of corticosterone when compared to reference values reported in the literature for this species. However, loggerhead turtles captured in pelagic longlines had lower levels of this hormone (Williard et al., 2015).

In addition, during forced submergence, circulatory adjustments cause severe hypoxia in some tissues, due to the redirection of blood flow and oxygen stores to essential organs (Berkson, 1966; Lutz and Bentley, 1985). Upon resurfacing, heart rate increases gradually, promptly restoring blood and oxygen flow to all tissues, and re-establishing their metabolic functions and oxygen stores. This exposure to hypoxia, and the rapid transitions between ischemia/reperfusion of tissues, leads to reactive oxygen species (ROS) production, and thus oxidative stress, which could contribute to oxidative cell damage (Hermes-Lima and Zenteno-Savın, 2002; Zenteno-Savin et al., 2002; Valdivia et al., 2007).

Depending on the extent of injuries suffered or stress experienced, these physiological responses can persist after the liberation from the nets, affecting the survival chances of these animals. Stabenau and Vietti (2003) observed that after being released, sea turtles spent long periods on the surface to rest and restore blood homeostasis to pre-submergence levels. This period may extend up to 20 h, determined by how long the turtle has been submerged and how often it has been exposed to this situation (Lutz and Dunbar-Cooper, 1987). Furthermore, the time spent on the surface can leave turtles vulnerable to other threats, such as predators and boat strikes (Snoddy et al., 2009).

In this context, biochemical assessment of blood, at the time the turtle is captured can provide valuable information about the animal's physiological condition and the best treatment after release from the nets. This evaluation can also help in decision-making to mitigate mortality and injuries suffered during the capture. However, to assess physiological data, it is necessary to compare results with baseline physiologic values that are considered normal for a given species. Therefore, these values can be used to confirm if the results obtained for turtles caught in fishing gears are altered from the normal physiological conditions. Since these parameters evaluate metabolic mechanisms and pathways in specific and short-term physiological situations, seasonal variations must be considered.

Due to the lack of studies about seasonality variations on blood biochemical of animals captured by different fishing artifacts, the present study aims to: 1) establish blood reference values for a population of juvenile *Chelonia mydas* from Ubatuba- Brazil; 2) evaluate the physiological effects of bycatch on gillnet and pound net, that are commonly used in the region, comparing the results with those from individuals captured by diving; 3) verify the seasonal variation of these parameters.

2. Material and methods

2.1. Study area and period

Ubatuba (23°26′S and 45°05′W) is located in the Northern Coast of São Paulo State, Brazil. Its coastline is about 100 km long and alternates between sandy beaches and rocky shores. Traditional communities, that have had artisanal fisheries as the first or the second main source of income, occupy many beaches in this region.

Ubatuba is also an important feeding area for juvenile green sea turtles in Brazil, and therefore many incidental captures occur because of the overlap between artisanal fisheries and the sea turtle's habitat (Gallo et al., 2006).

This study was conducted over two season periods, between January and March (summer) and from July to September of 2015 (winter). Data collection was authorized by Chico Mendes Institute for Biodiversity Conservation (ICMBio) through the special license number 45895, issued by the Biodiversity Authorization and Information System (SISBIO).

2.2. Capture methods

Diving: turtles were captured in shallow water (2 to 10 m deep) by swimmers using standard snorkel, masks, and fins. Once a turtle was sighted, it was chased by swimming, captured by hand, and then carried to the shore and placed on the beach for processing. This method was used to establish reference values to be compared with those from animals captured by artisanal fisheries, because direct capture represents only a mild stressor (Hunt et al., 2016).

Sea turtles were captured by fishermen by two methods during their daily activities in the region:

Uncovered Pound net: is a trap made of nets anchored to the bottom of the sea and extending through the water column. The nets are open at the surface. It is not a species-selective method and captured fishes stay alive and swim inside the walls formed by the nets. Likewise, turtles captured by this method usually do not entangle in the nets and can surface for breathing, staying alive until the fishermen come to check the nets, which occurs from 1 to 3 times a day; hence, the maximum time spent in the pound net by any given turtle would have been 12 h.

Gillnets: are usually placed in waters 2 to 15 m deep, by 1 to 3 fishermen using a canoe, and can operate on the surface, in midwater, or at the bottom. The nets are 50 to 100 m long, 1.5 to10 m high, and the mesh size is usually 10 to 14 cm. Gillnets stay, at most, 10 h in the water, until the arrival of the fishermen. Despite the long time, no turtle died in this method of capture during the study, and the time each turtle remained in the nets has not been estimated.

Fishermen were trained to manipulate the sea turtles by Projeto TAMAR. Stress generated by capture and handling can influence blood biochemistry; therefore, all turtles were handled in the same way and blood samples were obtained within approximately 5 min after releasing from the nets.

2.3. Sample collection

Each turtle was measured with a flexible plastic tape (to the nearest 0.1 cm) over the curved carapace length (CCL, nuchal notch to posterior tip of carapace) and the curved carapace width (CCW, widest points), according to Bolten (1999). Bodyweight (BW) was measured to the nearest 0.1 kg with a spring scale. Only juveniles (CCL $<$ 65 cm) were included in this study (Chaloupka and Limpus, 2005). As the sex of immature sea turtles cannot be established based on an external examination (Bolten and Bjorndal, 1992), and laparoscopy was not used to determine the sex with certainly, this parameter was not known.

To identify the individuals, turtles were double tagged (one tag was applied to each of the front flippers) with Inconel tags (National Band

and Tag Company, USA, style 681), according to Limpus (1992), before being released.

Turtle health status was determined using visual assessments (Labrada-Martagón et al., 2010). Turtles in good physical conditions (convex plastron), displaying no external lesions and/or injuries, and with no evidence of emaciation or disease (fibropapillomatosis), were classified as healthy. Turtles exhibiting clinical abnormalities, any external skin or carapace lesion, recent traumatic injuries (scars were discounted), flipper amputations, or obvious signs of illness (e.g., emaciation), were classified as unhealthy and were not used in this study.

Sea surface temperature data for the region where turtles were captured were obtained from the National Oceanic and Atmospheric Administration (http://www.noaa.gov/).

2.4. Blood collection and preparation

Blood samples (2 mL) were collected from the dorsal cervical sinus (Owens and Ruiz, 1980), with a 21G sterilized needle, and transferred into 4 mL lithium-heparin vacuum anticoagulant tubes (BD, Vacutainer®). Sample tubes were kept in a cooler with ice before their processing. Plasma was separated by centrifugation at 5000 rpm for 5 min, and subsequently frozen and stored at −20 °C until analyzed.

2.5. Plasma biochemistry

Plasma samples were analyzed in duplicate, using spectrophotometric techniques and commercial kits (Labtest®), according to the instructions of the manufacturer (Silva and Bianchini, 2019). The following kits were used: total proteins (Ref.: 99–250), glucose (Ref.: 133–1/500), lactate (Ref.: 138–1/50), triglycerides (Ref.: 87–2/100), total cholesterol (Ref.: 76–2/100), uric acid (Ref.: 140–1/250) and albumin (Ref.: 19). Low-Density Lipoprotein (VLDL) levels were calculated from triglycerides values using the formula: Triglycerides / 5 = VLDL (Goldberg et al., 2013).

Total lipids were measured in duplicate according to Frings and Dunn (1970), a colorimetric method based on the sulfo-phospho-vanillin reaction. The results were expressed as mg/dL.

2.6. Corticosterone

Plasma concentrations of corticosterone were determined using Corticosterone ELISA kit (ENZO Life Sciences®), according to the instructions of the manufacturer (ADI-901-097), with a limit of detection of 27 pg/mL (Santos et al., 2017). Samples were diluted 1:40 times in assay buffer provided in the ELISA kit. Standard curves were prepared in buffer with known amounts of corticosterone (160, 800, 4.000, 20.000 and 100.000 pg/mL). All samples were analyzed in duplicate and measured in a single assay to avoid inter-assay variation. The intra-assay coefficient of variation was 1.37%. Absorbance was read at 405 nm using a microplate reader.

Corticosterone concentration was determined by interpolation from the standard curve generated in the assay. The results were expressed as ng/mL.

2.7. Estimation of lipid peroxidation level

The lipid peroxidation was measured in the form of thiobarbituric acid reactive substances (TBARs) by the method of Wills (1966). In brief, 500 μL of 20% trichloroacetic acid was added to 125 μL of plasma and centrifuged at 3000 rpm for 10 min. Then, 500 μL of sulfuric acid and 500 μL of color reagent were mixed with the supernatant. The reaction mixture was incubated in a boiling water bath for 15 min and cooled to room temperature. Next, 1.75 mL of n-butanol was added to this mixture, which was centrifuged at 3000 rpm for 5 min. Peroxidation was measured as the production of malondialdehyde (MDA), which in combination with TBARS, forms a pink chromogen that can be measured at 530 nm. All samples were analyzed in duplicate and the results were expressed as nmol/MDA/mL.

2.8. Total antioxidant capacity assay (TAC)

The total antioxidant capacity was measured by trolox equivalent antioxidant capacity assay (TEAC) (Re et al., 1999), which is based on the inhibition by antioxidants of the absorbance of the radical cation of 2.2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)(ABTS).

The blue-green $ABTS^{\bullet}$ was produced through the reaction between 7 mM ABTS and 2.45 mM potassium persulfate in water. This solution was stored in the dark for 12–16 h before use.

The concentrated $ABTS^{\bullet+}$ solution was diluted with phosphate buffered saline 5 mM, pH 7.4 to a final absorbance of 0.70 (± 0.35) at 734 nm. Then, 3 mL of this solution was added to 30 μL of plasma, and the decrease in absorption at 734 nm was measured during 6 min after the mixture. The reaction rate was calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays. All samples were analyzed in duplicate and the assay results were expressed in mmol Trolox equivalent/l.

2.9. Statistical analysis

Data were summarized using means, standard deviation (SD), and range (minimum–maximum) values for each parameter. The Kolmogorov-Smirnov test was used to test for normality and Levene's test for equality of variances.

To assess differences between seasons (summer and winter) parametric (Student *t*-test) or nonparametric (Mann-Whitney *U* test) analyses were performed, as appropriate. ANOVA, with post hoc Bonferroni ($p > 0.05$) or Games-Howell ($p < 0.05$) pairwise comparisons, were used to estimate differences between capture methods (gillnets, pound net and diving), for data with equal variance and normality. Data failing to meet the assumptions of normality and equal variance were analyzed with Kruskal–Wallis, followed by Dunn's multiple comparison test.

The relationship between each blood parameter and CCL (as an indicator of body size), sea surface temperature, and corticosterone levels were evaluated using Pearson's correlation test (r) or Spearman's correlation test (ρ), depending on whether both concentrations exhibited a normal distribution. All results with a $p \leq 0.05$ were considered significant. Statistical Package for Social Science (SPSS) 20.0 was used to analyze the data.

3. Results

A total of 96 green sea turtles were captured during the summer and winter of 2015 (summer: 2 by gillnets, 19 by pound nets, and 12 by diving; winter: 34 by gillnets, 10 by pound nets, and 19 by diving). All of them were considered healthy by visual physical examination, and without fibropapillomatosis at the time of capture.

All green sea turtles caught were juveniles, with CCL between 31.5 and 68 cm (mean: 43.6 ± 8.87 cm), and CCW within the range of 28.5–63 cm (mean: 39.5 ± 7.4 cm). The body weight ranged between 3.5 and 34 kg (mean: 10.9 ± 7.5 kg). There were no significant differences between the size of the turtles and capture methods (CCL: $p = 0.070$; CCW: $p = 0.086$; Weight: $p = 0.092$) or seasons analyzed (CCL: $p = 0.165$; CCW: $p = 0.123$; Weight: $p = 0.160$).

Total protein ($r = 0.576$, $p = 0.0001$), glucose ($r = 0.230$, $p = 0.024$), albumin ($r = 0.524$, p = 0.0001), total lipids ($r = -0.336$, $p = 0.001$), triglycerides ($r = 0.334$, p = 0.001), and VLDL (r = 0.334, p = 0.001) correlated significantly with CCL.

Sea surface temperature at the collection sites/areas averaged 28.3 °C in the summer and 21.6 °C in the winter. Total protein (ρ = 0.681, p = 0.0001), glucose ($r = 0.380$, $p = 0.002$), albumin

Table 1
Blood reference values of juveniles *Chelonia mydas* in Ubatuba, Brazil, during summer and winter, and values found for the same species in Brazil.

Parameters	Summer			Winter			Reference values for other populations of juvenile green sea turtles (mean and range)
	n	Mean ± SD	Range	n	Mean ± SD	Range	
Glucose (mg/dL)*	12	114.69 ± 15.0	(86.91–141.20)	19	68.58 ± 12.12	(48.04–87.25)	89.3 (59.6–120.2)[a], 113 (102–125)[b]
Lactate (mmol/L)	12	3.52 ± 1.25	(1.87–5.62)	19	3.68 ± 1.46	(1.70–6.73)	–
Total proteins (g/dL)*	12	5.31 ± 2.29	(2.7–9.0)	19	2.32 ± 0.81	(0.95–3.46)	3.7 (1.3–5.5)[a], 3.20 (2.91–3.48)[b]
Uric acid (mg/dL)*	12	4.04 ± 1.0	(2.47–6.16)	19	2.46 ± 1.39	(0.71–6.91)	1.1 (0.0–2.5)[a], 1.99 (1.68–2.32)[b]
Albumin (g/dL)*	12	2.09 ± 0.41	(1.42–2.6)	19	1.18 ± 0.61	(0.20–2.43)	1.0 (0.1–1.7)[a], 0.93 (0.87–1.01)[b]
Total lipids (mg/dL)*	12	197.95 ± 46.44	(109.22–257.11)	19	318.21 ± 143.58	(138.23–573.38)	–
Triglycerides (mg/dL)	12	206.22 ± 142.53	(99.21–503.44)	19	199.61 ± 72.55	(37.50–306.03)	89.6 (11.3–209.8)[a], 215 (158–271)[b]
VLDL cholesterol (mg/dL)	12	41.24 ± 28.51	(19.84–100.69)	19	39.92 ± 14.51	(7.50–61.21)	–
Total cholesterol (mg/dL)	12	82.64 ± 33.23	(49.80–140.81)	19	88.99 ± 57.49	(15.0–223.0)	113.6 (15.0–212.7)[a], 202 (181–223)[b]
Corticosterone (ng/mL)	12	3.6 ± 2.37	(1.4–7.7)	19	3.0 ± 2.25	(0.9–8.5)	–
TEAC*	9	0.57 ± 0.02	(0.54–0.60)	14	0.55 ± 0.02	(0.50–0.58)	–
TBARS*	9	3.14 ± 0.80	(2.21–4.64)	14	2.70 ± 0.42	(2.12–3.37)	–

Asterisk (*) indicates significant difference ($p < 0.05$) between summer and winter.
[a] Santos et al. (2015) – Espírito Santo state, Brazil.
[b] Mello and Alvarez (2019) – São Paulo state, Brazil.

($r = 0.741$, p = 0.0001), uric acid ($\rho = 0.210$, $p = 0.039$), total lipids ($r = -0.680$, p = 0.0001), triglycerides ($r = 0.222$, $p = 0.029$), and VLDL (r = 0.222, p = 0.029) were significantly correlated with sea surface temperature.

3.1. Reference values

The results obtained for sea turtles caught by diving, during summer and winter, were considered as reference blood values and they are found in Table 1, as well as the values already established for other populations of juvenile green sea turtles in Brazil.

Turtles captured in summer had significantly higher levels of glucose ($t = 3.83$, $p < 0.002$), total proteins (U = 141, $p < 0.0001$), uric acid (U = 720, $p = 0.05$), albumin ($t = 11.83$, p < 0.002), TEAC ($t = 2.58$, $p = 0.012$), and TBARS ($t = 3.18$, $p = 0.0023$) compared with turtles captured in the winter. In contrast, total lipids levels were significantly lower during the summer than during the winter ($t = 7.91$, p = 0.002).

There were no significant differences in lactate (U = 1067, $p = 0.976$), triglycerides ($t = 1.90$, $p = 0.059$), VLDL ($t = 1.91$, $p = 0.060$), total cholesterol ($t = 0.51$, $p = 0.610$), and corticosterone (U = 598, $p = 0.628$) levels between seasons.

Corticosterone was positively correlated with glucose ($\rho = 0.237$, $p = 0.041$), lactate ($\rho = 0.295$, $p = 0.010$), total lipids ($\rho = 0.252$, $p = 0.029$), and total cholesterol ($\rho = 0.280$, = 0.015); and negatively correlated with albumin ($\rho = -0.238$, $p = 0.040$).

3.2. Differences between direct capture and fisheries gear

Results obtained for sea turtles captured by gillnet, pound net and diving, during summer, and winter are found in Fig. 1.

In the two seasons, turtles entangled in gillnets had significant higher glucose, lactate and corticosterone levels than the other methods ($F_{(2,84)} = 20.11$, $p < 0.001$; H = 48.86, $p < 0.0001$; H = 36.89, p < 0.0001, respectively). They also had significantly lower levels of triglycerides ($F_{(2,91)} = 1.14$, $p = 0.011$) and VLDL cholesterol ($F_{(2,91)} = 1.15$, p = 0.012), and higher levels of total proteins, uric acid, and total cholesterol than those observed for turtles captured by diving (H = 15.48, $p = 0.0017$; H = 15.62, $p = 0.003$; $F_{(2,94)} = 16.02$, p < 0.0001, respectively). In the winter, turtles caught by gillnets had significant lower levels of albumin and higher levels of total lipids compared to the other capture methods ($F_{(2,94)} = 15.32$, $p = 0.0004$; $F_{(2,91)} = 3.949$, p = 0.02).

Individuals caught in pound nets had significantly higher levels of glucose, lactate, corticosterone, and cholesterol than turtles captured by diving ($F_{(2,84)} = 20.11$, p < 0.001; H = 48.86, $p = 0.004$; H = 36.89, p = 0.001; $F_{(2,84)} = 16.02$, p < 0.0001, respectively). Seasonal differences in sea turtles captured by pound net were higher total protein and TBARS levels during the summer (H = 15.48, $p = 0.0019$, $F_{(2,63)} = 6.23$, $p = 0.0034$, respectively), and lower levels of uric acid, in the winter (U = 15.62, $p = 0.03$).

Even though the number of turtles captured by gillnets during the summer was too small to perform statistical analysis, the values found for these animals are within the pattern exhibited by turtles caught by other collection techniques during the seasons. Therefore, they are represented in the graphs and were included in the statistical analysis.

4. Discussion

Some research groups have reported blood biochemistry values of sea turtles around the world. All authors agree that baseline values should be established for healthy sea turtles, at the population level and by geographic area, considering disease status, age, sex, and seasonal variations (Aguirre and Balazs, 2000; Stamper and Harms, 2005; Whiting et al., 2007; Prieto-Torres et al., 2013), to be able to compare with diseased or stressed animals. This is the first study to verify the influence of seasonality on the biochemical parameters for a population of juvenile green sea turtles on the southeastern coast of Brazil.

Several studies concluded that the biochemical reference intervals (RIs) in sea turtles must be considered regionally (Deem et al., 2009; Flint et al., 2010); therefore, comparisons between the results of this study and previous studies are complicated and possibly inaccurate, because the species behavior (Samour et al., 1998), foraging areas (Whiting et al., 2007), and methods of blood collection, handling, processing, and biochemical analysis that must be a source of possible variation (Bolten and Bjorndal, 1992). Thus, results from this study were compared with juvenile green sea turtles from Brazil to confirm that the levels observed were within normal ranges. Only two studies (Santos et al., 2015; Mello and Alvarez, 2019) that analyzed the same parameters were found, one of them also sampled in São Paulo state and the other in Espírito Santo state.

In general, the levels of most parameters analyzed in this work are like those found for juveniles of the same species in Brazil (Table 1). However, the average concentration of glucose (winter), total proteins (winter) and total cholesterol are below the mean values found by Santos et al. (2015) and Mello and Alvarez (2019). Nevertheless, they are between the range of quantified values. In contrast, the mean concentrations of total proteins (summer) and uric acid are above the

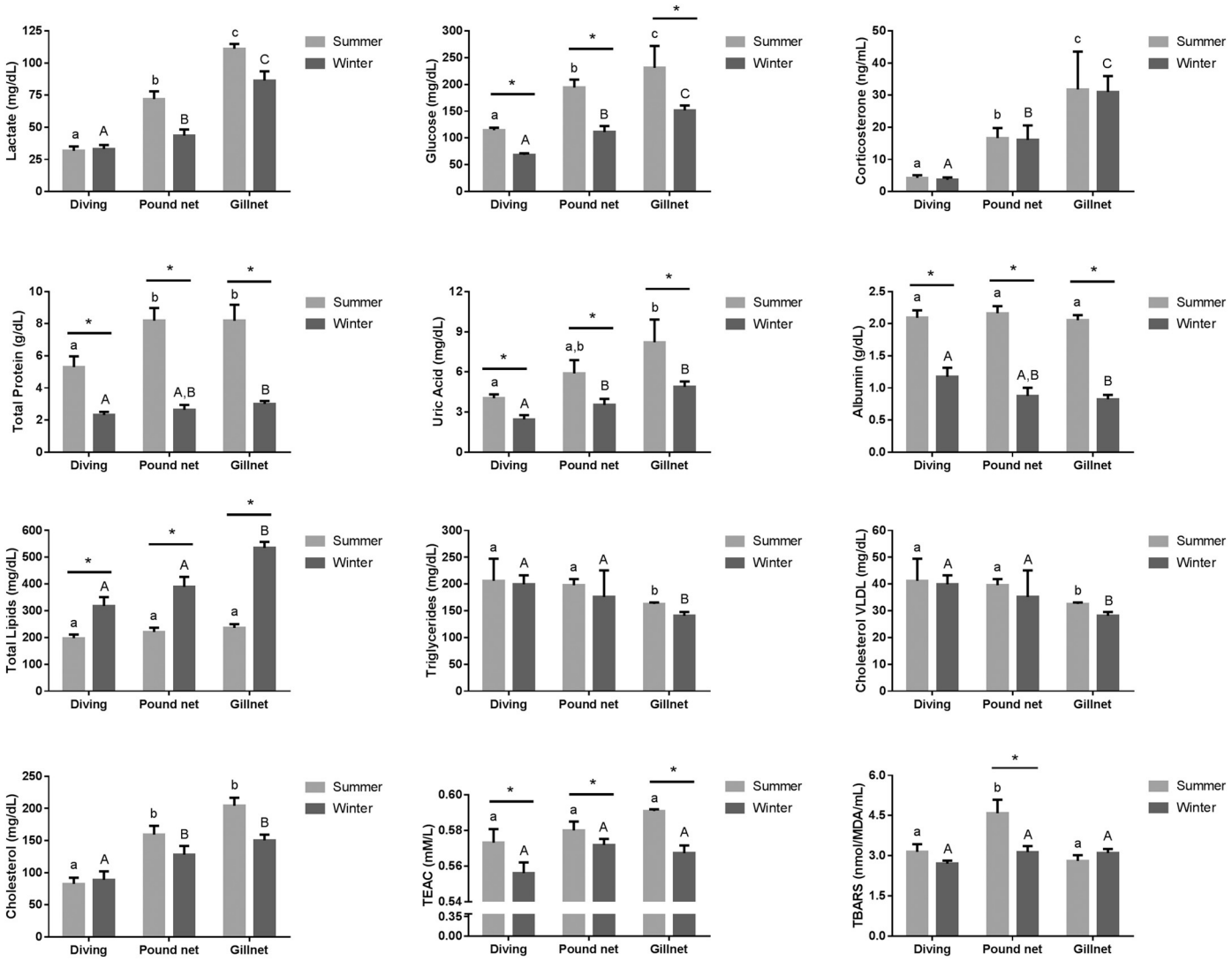

Fig. 1. Biochemical parameters (mean ± SD) found in juvenile *Chelonia mydas* captured by diving, pound net, and gillnet, during summer and winter. Different letters indicate significant different mean concentration values ($p < 0.05$) among capture methods in the summer (lowercase letters) and in the winter (capital letters). Asterisk (*) indicates significant different mean concentration between seasons ($p < 0.05$).

values already established by other authors, but also within the limit range of observed values.

Seasonality had no influence on corticosterone concentrations, confirming the hypothesis that this hormone is not primordial during daily and seasonal processes, where its basal concentrations can regulate the behavior and physiology of the animal, without inducing an emergency response (Landys et al., 2006). It is known that migration, reproductive process, and diseases can cause changes in corticosterone levels in sea turtles (Aguirre et al., 1995; Jessop, 2001; Hamann et al., 2002; Jessop et al., 2002), which often increase its levels above baseline values. However, the population evaluated in this study is composed of healthy and resident juvenile individuals, which may be another factor in which corticosterone levels did not present seasonal influences.

Green sea turtles captured in the summer showed higher glucose concentration than those captured in the winter. Elevation of glucose levels can be attributed to increased glucocorticoids (stress) during the capture (Gregory and Schmid, 2001). But corticosterone concentration remained unchanged in the two seasons analyzed, indicating that this hormone can increase glucose temporarily but not seasonally.

However, increased glucose levels along with higher uric acid values may be associated with increased carbohydrate and protein intake, respectively, during the summer (Labrada-Martagón et al., 2010). Green sea turtles are considered foragers and are expected to have a predominantly herbivorous diet (Mortimer, 1982) but their diet may vary according to the availability of food in their environment. When sea grass and algae are scarce, they can feed on mollusks, crustaceans, and other animals (Garnett et al., 1985).

Uric acid may be an indicator of increased catabolism/protein digestion in reptiles (Maixner et al., 1987), so the results of the present study (increased total protein levels and albumin, in the summer) may be associated with an omnivorous diet and an environment with a high availability and variety of food. Labrada-Martagón et al. (2010) reported the same seasonal pattern of total protein and glucose in juvenile green sea turtles in Mexico and concluded that, during the summer, the individuals had better nutritional conditions due to the greater food availability and/or better food quality.

The lower levels of total protein, albumin and glucose, in the winter, may be related to the decrease in metabolic rate, food availability, and/or water temperature (Moon et al., 1999). Green turtles submitted to cold water (simulating winter conditions) presented decreased values of total protein, that were justified by the lower feed intake under these conditions in which metabolic rate is decreased (Southwood et al., 2003a). Similar to this result, Osborne et al. (2010) found a relationship between water temperature and plasma protein concentration, showing

that in colder months sea turtles have low protein levels. Likewise, in this study, a positive correlation was found between water temperature and glucose, total protein, uric acid, and albumin levels; and a negative correlation with total lipid levels.

Bonnet (1979) observed that juvenile green turtles that stayed 5 days without feeding had low levels of glucose and proteins, and high levels of lipids, a similar pattern was found in this study during the winter. Therefore, water temperature along with decreased metabolism and food availability may be influencing the concentrations of these metabolites in the population of juvenile green sea turtles from Ubatuba.

In sea turtles, lipids are stored in the subcutaneous layers and as visceral fat (Derickson, 1976; Kwan, 1994). This provides a stock of energy that can be mobilized to maintain metabolic processes during low supplies of food and/or reproduction, particularly in the period of the synthesis of sex hormones, gametogenesis, and vitellogenesis (Derickson, 1976; Kwan, 1994; Hamann et al., 2002, 2003). The increase in total lipid concentrations, observed in the present study in the winter, may be associated with energy losses due to a reduction in food supply and/or foraging activity (Koch et al., 2007), leading to a change in the metabolic substrate and inducing the catabolism of energy reserves. The reduction in total protein, albumin, and glucose values, along with a possible decrease in the metabolic rate already verified by other authors (Southwood et al., 2003a), corroborates this hypothesis.

Field observations and remote monitoring studies indicate that juvenile green turtles remain active during the winter; however, significant differences in diving patterns may occur (Mendonca, 1983; Southwood et al., 2003b). In Gulf of California, for example, green sea turtles are seldom, if ever, seen to surface during windy weather, and surface only rarely on sunny, calm days, during the winter. They can also be found overwintering on the sea bottom (Felger et al., 1976). In Florida, juvenile green turtles increase their daily movements, feed in deeper water, and spend less time foraging during colder months (18 °C) (Mendonca, 1983). On the other hand, in Australia, juveniles of *Chelonia mydas* spend more time in shallow waters and have longer dives during the winter (21.3 °C) (Southwood et al., 2003b).

When the individuals for the present study were captured, it was observed that they remain active in the colder months (21.6 °C), but they were not followed throughout the winter. However, low levels and non-lactate variation during seasons suggest that these animals are not using the anaerobic pathway for ATP production in their daily activities. Since long dives are associated with significant increases in plasma lactate (Lutcavage et al., 1989), the basal values of this metabolite do not undergo seasonal changes in this population.

Comparing the results obtained (Fig. 1), it can be observed that turtles caught in gillnets presented a significant 4-fold increase in lactate levels (summer: 12.33 mmol/L, winter: 9.58 mmol/L) compared to the baseline values obtained by diving (summer: 3.52 mmol/L, winter: 3.68 mmol/L). While turtles caught in pound nets had 2-fold higher concentrations of this metabolite (summer: 7.99 mmol/L, winter: 4.83 mmol/L).

These increases were also verified by Harms et al. (2003), who evaluated the effects of trawl and pound nets on loggerhead turtles and reported lactate values of 15.8 mmol/L and 1.3 mmol/L, respectively, after capture. Green sea turtles captured in gillnets also had high lactate values (30.6 mmol/L) (Snoddy et al., 2009), as well as loggerhead turtles caught in longlines (7.2 mmol/L) (Williard et al., 2015).

These differences between methods can be explained by the fact that capture by gillnet and trawl, often results in forced submergence, since the turtles become entangled, something that does not happen during the capture by the pound net and longline, because it allows free access to the surface for the turtle to breath. However, turtles caught in pound nets, make continuous and variable attempts to escape from nets (Harms et al., 2003), and this can cause lactate elevations in the plasma due to the activation of anaerobic metabolism.

The increase of lactate is indicative of a metabolic acidosis caused by forced submersion (hypoxia) and intense fighting, which results in a change from aerobic to anaerobic respiration (Stabenau et al., 1991; Hoopes et al., 2000; Stabenau and Vietti, 2003; Snoddy et al., 2009). During hypoxia, energy production in the absence of oxygen is through anaerobic glycolysis, which uses glucose as the substrate, raising levels of this metabolite in the blood (Clark and Miller, 1973). The high glucose rate in this situation maintains the levels of ATP and ionic homeostasis in the brain, reducing the metabolic demand to a level attended by anaerobic glycolysis and increasing the time of tolerance to hypoxia and/or anoxia (Lutz and Bentley, 1985; Lutcavage and Lutz, 1997).

In the present study, glucose levels had the same pattern as lactate levels. Turtles captured in gillnet and pound net showed higher concentrations of glucose (summer: gilnet: 231.04 mg/dL, pound net: 194.34 mg/dL; winter: gillnet: 151.71 mg/dL, pound net: 111.05 mg/dL) than the baseline values (summer: 114.69 mg/dL, winter: 68.58 mg/dL). These increases in glucose levels suggest that the species is mobilizing glycogen stores to obtain ATP through anaerobic metabolism (Clark and Miller, 1973). Hyperglycemia has also been reported in studies on anaerobic metabolism in *Pseudemys scripta elegans* (Clark and Miller, 1973) and *Chrysemys picta* (Keiver et al., 1992) submitted to forced diving.

Elevated glucose levels may also be associated with induction of a systemic response to stress (Wingfield et al., 1998). Aguirre et al. (1995), Gregory et al. (1996), and Snoddy et al. (2009) reported high levels of glucose and corticosterone in turtles subjected to handling and capture stress. As already mentioned, the turtles captured in this study (gillnet and pound net) had high levels of glucose, which were similar to the pattern obtained for corticosterone. Entanglement-netting produced the highest values of corticosterone (summer: 36.8 ng/mL, winter: 31.0 ng/mL), while pound net capture had a moderate elevation (summer: 17.6 ng/mL, winter: 16.1 ng/mL), compared to baseline concentrations (diving: summer: 3.6 ng/mL, winter: 3.0 ng/mL).

In stress situations, glucocorticoids together with catecholamines will provoke metabolic alterations in order to mobilize and supply energy to the body through the lipolysis, proteolysis, and gluconeogenesis to reestablish homeostasis (Jacob and Oomen, 1992; Wingfield et al., 1998). Thus, high levels of total protein and uric acid found in turtles captured in gillnet and pound net may be associated with the induction of protein catabolism by corticosterone.

Total lipid levels were also expected to be elevated, however, the concentrations of total lipids, triglycerides, and VLDL cholesterol were lower than, or equal to, those found for reference values. The decrease in triglyceride levels, together with lower VLDL cholesterol levels, may be associated with its increased uptake by other tissues, especially the skeletal muscles to produce energy, and/or by the decrease/absence of food activity during the capture period. Innis et al. (2010) also reported reduced levels of triglycerides in leatherback turtles (*Dermochelys coriacea*) caught by fishing artifacts and believed that this decrease is related to the fact that turtles do not feed during entanglement. In contrast, elevated levels of total cholesterol in animals caught in this study may be related to its mobilization in plasma for the synthesis of corticosterone (Tóth et al., 1997).

The difference in antioxidant activity between seasons may reflect the quantity and quality of food in the summer, resulting in animals with better fitness. Roark et al. (2009) showed that ad libitum-fed turtles demonstrated elevated antioxidant function relative to continuously food-restricted turtles. It is known that the antioxidant capacity of aquatic turtles is considered to be exceptional among ectothermic vertebrates (Rice et al., 2002; Willmore and Storey, 1997). Because of their adaptations to drastic changes in oxygen availability which results in a high constitutive antioxidant capacity (Wilhelm et al., 2002; Hermes-Lima and Zenteno-Savın, 2002).

Baker et al. (2007) evaluated the antioxidant capacity of hatchling *Chrysemys picta*, following anoxia, and found no differences in experimental and control animals. However, lactate levels increase in

response to hypoxia. The same pattern was observed in this study regarding the capture method (no difference in antioxidant activity, but high lactate levels in turtles caught in pound net and gillnet). This strategy provides a high constitutive level of antioxidant defenses that would serve to prevent or buffer damage, due to bursts of reactive oxygen species generation during reoxygenation after submergence.

Despite the extraordinary antioxidant capacity of *Chelonia mydas*, oxidative stress may nevertheless present a problem for these animals during capture. Animals that were caught in pound nets, but not in gillnets, sustained oxidative damage to plasma lipids (TBARS) in the summer. These differences between methods can be explained, at least in part, by the contribution of non-enzymatic antioxidant defense that were not evaluated in this study. Another potential limitation is the lower number of animals captured by gillnet in the summer, which may not have been sufficient to show oxidative damage. In the winter, there were no differences between methods regarding TBARS levels. Nevertheless, uric acid levels were higher in animals captured by fishing artifacts, which could explain the lower levels of oxidative damage in these turtles because of its potent radical scavenger.

In the present study, it was expected that the differences in biochemical parameters between turtles caught in pound net and diving would be minimal. Although the pound net is a passive capture mechanism, allowing free access to the surface to breathe (Harms et al., 2003), seven of the twelve parameters evaluated were significantly different from the values obtained for animals captured by diving. Capturing in gillnets caused more disturbances than the pound net, in which all the parameters evaluated differed significantly from the basal values obtained for the species, in the same study area, and in the same period.

The use of biochemical parameters to evaluate sea turtle health has important applications for rehabilitation of ill and injured sea turtles as well as for assessment of the health status of free ranging sea turtle populations. This is the first study to evaluate the physiological effects of incidental capture by gillnet and pound net, and to compare them with established reference values for juvenile green sea turtles in the same area and over the same period, considering seasonal variation. Reference values found can serve as baseline information for monitoring the impacts of various disturbances on the population, guiding management of clinical cases in the rehabilitation setting, and establishing criteria for evaluating the prognosis for release of turtles from rehabilitation centers. The results obtained by gillnet and pound net can be considered for conservation projects, in areas where these fishing artifacts occur, to assist in the decision-making regarding the immediate release or rehabilitation of the animal, and in the mitigation of mortality and insults caused by these artifacts.

Additionally, researchers that uses pound net and gillnet to establish references values for a population must be careful in the interpretation of the results. Because as we could see they can alter the normal metabolism of the animal and thus influence the concentration of the evaluated metabolites. In these cases, animals should stay the minimal time as possible in the fisheries gear to have minimal physiologic alterations. As already seen, seasonality also plays an important role in the intermediate metabolism of the animals. Its evaluation during the establishment of reference values for a species must be indispensable.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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