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Health condition of *Chelonia mydas* from a foraging area affected by the tailings of a collapsed dam in southeast Brazil

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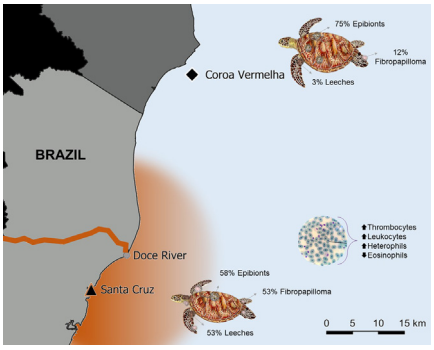
HIGHLIGHTS

- A dam collapsed in southeast Brazil affecting a foraging area (Santa Cruz).
- Sea turtles are less healthy, with higher prevalence of fibropapillomatosis and ectoparasites.
- They also had a worse nutritional status and higher degree of hepatic and renal lesions.
- Santa Cruz turtles also presented elevated levels of As and Cu.

GRAPHICAL ABSTRACT



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ABSTRACT

In 2015, the failure of the Fundão dam caused the release of 43 million m^3 of tailings into the Doce River Basin, in southeast Brazil. It was considered the largest environmental disaster of the world mining industry. The tailings, composed mostly of heavy metals, caused massive destruction of the Doce River ecosystem endangering the organisms that live in the coastal zone where the mud reached the ocean. Among the exposed species are the sea turtles that use the region for food. The aim of this study was to evaluate the effect of contaminants on the health status of juvenile green sea turtles that feed in a coastal area exposed to ore mud (Santa Cruz) and to compare them with animals from an area not directly affected (Coroa Vermelha). A physical examination was performed to determine the health status. Blood samples were analyzed for hematological and biochemical parameters, and metal concentrations (As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, and Zn). Santa Cruz sea turtles had more ectoparasites and a higher incidence of fibropapillomatosis. Statistically significant differences between sites were found for levels of calcium, phosphorus, glucose, protein, albumin, globulin, cholesterol, triglycerides, urea, CPK, ALT, and AST. The count of leukocytes, thrombocytes, and heterophils, as well as the concentrations of As and Cu were higher in Santa Cruz turtles. Together the results show a worse nutritional status and a greater degree of liver and kidney damage in animals affected by the tailings. The health status may indicate a physiological deficit that can affect their immune system and behavior, which is supported by the higher fibropapillomatosis tumor score and ectoparasite load in these animals. These results support the need for long-term monitoring of the exposed area to quantify the direct and indirect influence of the heavy metals levels on sea turtles and how this reflects the environmental health.

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1. Introduction

In 2015, the collapse of Fundão dam in Mariana (Minas Gerais state, Brazil) released 43 million cubic meters of tailings (80% of the total contained volume) from iron ore extraction. The mud engulfed the nearest village Bento Rodrigues displacing its entire population (six hundred citizens), killing nineteen people, and damaging cultural heritage dating to the 1700s (MB, 2016). On the same day of the accident, the tailings reached the Doce river basin that has 98% of its area within the Atlantic Forest biome, which is considered one of the world's most important hotspot for biodiversity conservation due to high levels of species richness and endemism (Myers et al., 2000).

The tailings were composed mostly of iron ore and silica, and contained high levels of arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), manganese (Mn), nickel (Ni), and selenium (Se) (IGAM, 2015; MB, 2016). The "mud tsunami" killed thousands of fish, invertebrates, and benthic organisms along its path, causing drastic environmental damage to watercourses in Doce river basin and associated ecosystems (Samarco, 2016). The residents of riverside communities (included indigenous populations) were left without access to clean water, food, fishing resources, crop production sites, hydroelectric power generation, and commodities that support the local economy (Fernandes et al., 2016). The mudflow spread along a 668 km trajectory making it the largest environmental disaster in the world mining industry, both in terms of tailings volume dumped and the geographic extent of socioeconomic and environmental damage (Carmo et al., 2017; Samarco, 2016).

Sixteen days after the accident, the tailings reached the Atlantic Ocean, endangering organisms living in the coastal zone (Escobar, 2015). In the sea, the river plume dispersed mostly southward, and the main driver was the wind along with the presence of the Brazil Current. Marta-Almeida et al. (2016) modeled the dispersion pattern of the plume and showed that Abrolhos Marine National Park, an important protected area situated 200 km north of Doce river was not directly affected by the disaster. In contrast, three conservation units situated towards the south of the river mouth were right in the most affected region core: the Comboios Biological Reserve at the Doce river mouth, the Santa Cruz National Wildlife Refuge, and the Costa das Algas Environmental Protection Area (38 km from the Doce river mouth). Costa das Algas Environment Protection Area together with Santa Cruz National Wildlife Refuge presents a wide variety of calcareous or non-calcareous marine macroalgae, considered the richest macroalgae flora in Brazil (Gastão et al., 2020). They provide substrate, shelter, and food for marine fauna, including sea turtles.

Marine macroalgae communities are affected by contaminants and nutrients due to their fast uptake of water-borne pollutants and their reliance on specific benthic habitat types for propagation and growth (Murray and Littler, 1978). It impairs physiological performance, growth rates, and biochemical diversity of macroalgae, and may also promote the loss of molecules such as enzymes. Consequently, it can result in community structure changes favoring opportunistic and more resistant species and excluding late successional and fragile ones (Murray and Littler, 1978). The decreased macroalgae species richness could reduce the dietary quality of generalist marine herbivores, such as green sea turtles, and a restricted diet may adversely affect sea turtles since different food items are required to optimize different life cycle processes such as growth, survival, and fecundity (Worm et al., 2006).

Among sea turtles that make use of coastal areas, is the green sea turtle (*C. mydas*) which inhabits nearshore waters close to human populations and enters river and lake estuaries (Limpus, 2008; Musick and Limpus, 1997). In these neritic zones, *C. mydas* primarily consume seagrasses and algae (Bjorndal, 1997) but they can also eat animal material when available (Bjorndal, 1997; Read and Limpus, 2002) and mangrove fruit when in season (Limpus and Limpus, 2000; Read and Limpus, 2002). Satellite tracking studies reveal that *C. mydas* exhibit high site fidelity to feeding areas, undertaking short term movements of 2 to 24 km (Limpus, 2008) taking up residency in these areas over decades (Chaloupka, 2004), making them susceptible to anthropic impacts from these areas for prolonged periods of their life. Thus, the coastal habit along with the high site fidelity makes them particularly prone to exposure to pollutants such as heavy metals, pesticides, and other contaminants that are washed into coastal waters.

Marine turtles readily accumulate environmental pollutants through a diversity of mechanisms such as dietary uptake or direct contact (with sediment and water) (Anan et al., 2001). Consequently, the contamination and composition of the turtles' diet can represent, to some extent, the state of the environment where they feed (Santos et al., 2011). In these animals, heavy metals bioaccumulation is critical, due to their high rates of daily consumption, long periods of time spent in coastal feeding areas near sources of pollution, and long-life spans (Miguel and Santos, 2019).

To better understand the effects that contaminant exposure and other threatening processes may impose on sea turtles, clinical evaluation is a useful tool. The alterations of clinical-laboratory parameters are early detected and very sensitive, thus assisting in the detection of possible additional effects that an organ or individual could suffer (Stegeman et al., 1992). Clinical health assessments usually include a physical examination and measurements of hematological and biochemical parameters. Some studies with sea turtles have correlated the levels of contaminants with health parameters (Day et al., 2007; Komoroske et al., 2011; Camacho et al., 2013; Álvarez-Varas et al., 2017; Cortés-Gómez et al., 2017; Ley-Quiñónez et al., 2017; Perrault et al., 2017; Tauer et al., 2017; Cortés-Gómez et al., 2018a), fibropapilloma tumors (Silva et al., 2016; Prioste, 2016), oxidative stress (Labrada-Martagón et al., 2011; Silva et al., 2016; Cortés-Gómez et al., 2018b), and carapace asymmetry (Cortés-Gómez et al., 2018c). Potential consequences of pollutants in sea turtles range from death to less obvious chronic and acute sublethal effects encompassing some diseases, like fibropapillomatosis.

Scientific research concerning the interactions of heavy metals with biochemical and physiological processes is extremely important to determine the impact on the health and survival of sea turtles in different life stages (Van de Merwe, 2008) which will influence population levels. Currently, the impact of the Fundão dam rupture tailings in Brazilian sea turtles is unknown. The objectives of this study are: (i) to assess heavy metal concentrations in the blood of green sea turtles feeding in an area affected by mining waste (Santa Cruz) and in a close area that was not directly affected by the tailings (Coroa Vermelha); (ii) to compare green sea turtle health reference intervals within the study areas using biochemical parameters, hematology, and in-field visual indicators of health (barnacles and ectoparasites presence, eye lesions, fibropapilloma tumors, and body condition scores); and (iii) to correlate green sea turtle health parameters with heavy metal levels to help determine whether contaminant exposure adversely affected sea turtle health at individual and populational levels.

2. Material and methods

2.1. Study sites

Coroa Vermelha reef (CV) is distant from anthropogenic sources, located approximately 15 km from the municipality of Nova Viçosa, on the southern coast of Bahia state, Brazil (17° 50′ S 39° 10′ W) (Fig. 1). It has an elongated form surrounded by small, isolated coral pinnacles, on its top there are many shallow-water reef species such as zoanthids, macroalgae, and turf, as well as gastropod vermetids and coralline algae. One species of hydrocoral and seven species of coral were identified along the CV reef: *Mussismilia harttii*, *Mussismilia hispida*, *Mussismilia braziliensis*, *Agaricia* spp., *Favia* spp., *Siderastrea* spp., *Porites* spp. and the hydrocoral *Millepora alcicornis* (Vasconcelos, 2014). In the southern part of the reef top, there is a small island built by carbonate sand formed from skeletal fragments of reef organisms, where reddish micro gastropod shells predominate, which probably contributed to the name of the reef. This small island is about 1 m above sea level, with sparse vegetation (Silva et al., 2013b).

Santa Cruz district (SC) is in the municipality of Aracruz, northern Espírito Santo state, Brazil (19° 49′ 06″ S e 40° 9′ 31″) (Fig. 1). Santa Cruz was founded at the Piraquê-mirim and Piraquê-açu river's mouth, being

Fig. 1. Map of study areas in Brazil. Santa Cruz, Espírito Santo state, directly affected by the tailings and Coroa Vermelha, Bahia state, not directly affected by the tailings.

inhabited by indigenous people and traditional communities such as artisanal fishermen and shellfish collectors. The rivers meet and jointly flow to the Atlantic Ocean forming a large estuarine complex, bordered by an extensive mangrove. The municipality coastline is part of the Costa das Algas Environmental Protection Area, which also encompasses the Santa Cruz National Wildlife Refuge. It has wide calcareous algae seabed and non-calcareous macroalgal biodiversity, as well as biolitoclastic and litoclastic sediments, forming a mosaic of seabed environments (Gastão et al., 2020) that provides an important habitat for benthic, pelagic, and demersal fauna, including commercial and threatened fish species in southeastern Brazil. It is also recognized as a green turtle pasture area (IBAMA, 2006). Santa Cruz seabed is a considerable part of the litoclastic sediments originate from the Doce river fluvial system (Silva et al., 2013a).

The Santa Cruz area was directly impacted by the primary and secondary plumes of tailing sediments from Doce river, which was visually evident. Coroa Vermelha, however, was not directly impacted in the acute and chronic phases, receiving secondary and tertiary plume according to coastal transport modeling (Magris et al., 2019), without visual signs of its presence (Francini-Filho et al., 2019).

2.2. Turtle capture and sampling

Between 2018 and 2019, green sea turtles (*C. mydas*) were captured using monofilament nylon gillnets (8 cm mesh size of 200 m stretched), for one week, four months per year (February, April, July, and November). Nets were set parallel to the sea current, for 8 h and monitored constantly every 30 min to avoid the drowning of any entangled animal. No sea turtle was harmed during the study. Captured turtles were brought on board for blood sampling and health assessment.

Blood collection was performed before all other procedures to avoid stress bias in blood test values. Samples were taken by venipuncture from the cervical venous sinus (Owens and Ruiz, 1980) following manual restraint, using 21gauge sterilized needles and 5 mL or 10 mL syringes. The blood volume collected was calculated based on the statement that sea turtle blood volume is 5 to 8% of its body weight and the maximum blood volume to be obtained from a turtle can be up to 0.7% of its weight (Jacobson, 1993). Six blood smears were immediately made from fresh blood and air-dried. Blood samples were transferred into Vacutainer© (Greiner Bio-one, Brazil) tubes containing lithium heparin to subsequent plasma biochemistry and complete blood count, or Vacuette® Trace Elements Sodium Heparin (Greiner Bio-one, Brazil) tubes for heavy metals quantification. Tubes were kept in a cooler with ice packs until processed at the field station laboratory within 6 h of collection. Direct contact of ice and blood tubes was prevented to avoid hemolysis. In the field station laboratory, samples were evaluated (hematology) or separated for further analysis.

The study was performed under the license of Biodiversity Authorization and Information System from Chico Mendes Biodiversity Institute (SISBIO/ICMBio), number 61063.

2.3. Morphometrics and health assessment

Prior to turtle release, each sea turtle was measured over the curved carapace length (CCL, nuchal notch to posterior tip of carapace) and the curved carapace width (CCW, widest points) with a flexible plastic tape (to the nearest 0.1 cm) (Bolten, 1999). Bodyweight was measured with a spring scale (to the nearest 0.1 kg). Body condition index (BCI) was used as an indirect predictor of the nutritional status and/or energy reserves in sea turtles. It was calculated according to the formula BCI = [weight (kg) / SCL^3 (m)] × 10,000 (Bjorndal et al., 2000). Because sex determination is not feasible in juveniles based on phenotypic parameters, sex identification was not performed. To identify the sea turtles, one Inconel tag (National Band and Tag Company, USA, style 681) was applied to each of the front flippers, according to Limpus (1992), supplied by Fundação Pró-TAMAR. Following these procedures, individuals underwent a physical examination to establish the body condition (BC) including a visual evaluation of the chest musculature, the presence of fat in the cervical musculature and the neck, and the depression of plastron concavity (Thomson et al., 2009). Based on these criteria, BC was scored as good (3), average (2), or poor (1) (Walsh, 1999).

Several external health indicators were also recorded for each captured turtle, including injuries and obvious signs of illness (i.e., emaciation, lethargy), percentage of carapace and plastron surface covered by barnacles, ectoparasite count, eye lesions, and fibropapillomatosis tumors. Some authors considered epibiont load as an indicator of physically compromised turtles (Deem et al., 2009). Thus, epibiont loads were estimated and categorized using the ordinal scale of 1 to 3 (1 = mild [until 30% of the carapace

covered by epibionts]; 2 = moderate [30 to 60% of the carapace covered by epibionts]; 3 = heavy [more than 60% of the carapace covered by epibionts]). Ectoparasites (mainly leaches and isopods) presence were also categorized using the ordinal scale of 1 to 3 (1 = mild [<10 ectoparasites]; 2 = moderate [10 to 20 ectoparasites]; 3 = heavy [>20 ectoparasites]). Animals with fibropapillomatosis were assigned a tumor score that considers tumors number and size (A (<1 cm), B (1–4 cm), C (>4–10 cm), and D (>10 cm)), according to Work and Balazs (1999). Each size category of tumors were counted separately for each anatomical region and the total number of tumors was classified as mild, moderate, and severe, according to the South-west Atlantic Fibropapillomatosis Score (FPSSWA) (Rossi et al., 2016). To avoid inter-observer variation, the same researcher examined the sea turtles during the entire study period.

2.4. Hematology

Traditional manual counting of blood cells was performed diluting whole blood in Natt-Herrick solution, and a Neubauer counting chamber (Neubauer chamber, New Optics, São Paulo, Brazil) was used to obtain total white blood cell (WBC) and red blood cell (RBC) count (Campbell, 2015). Differential leukocyte count was performed on blood smears using Instant-Prov Kit® (Newprov, Brazil), a Romanovsky-like staining. Heterophils, monocytes, eosinophils, lymphocytes, and basophils were differentiated out of 100 cells counted based on the morphological features. Blood smears were also screened for hemoparasites. To maximize consistency, hematology was performed by a single veterinarian familiar with sea turtle hematology.

Hematocrit (Hct) was obtained by measuring packed cell percentage using StatSpin® Microhematocrit Tubes (IRIS USA, Inc.). Tubes were spun for 5 min at 12,000 rpm in a centrifuge (XC-LED12K; Bio Lion). Using a metric ruler (StatSpin®; IRIS USA, Inc.), the column length formed by sedimented RBCs was measured in the capillary tube in percentage to the total length occupied by the blood that fills the capillary. Hemoglobin (Hb) was determined by commercial Labtest® Kit in a spectrophotometer (Quimis Q898DPT, Brazil), after centrifugation of the solution to at 5000 rpm for 5 min (Centribio® 80-2B, Equipar, Brazil) to remove the free red cell nuclei following red cell lysis. The erythrocytic index (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)) were calculated from the total erythrocytes count, hemoglobin, and hematocrit, according to Campbell (2015).

2.5. Plasma biochemistry

Plasma was separated by centrifugation at 5000 rpm for 5 min (Centribio® 80-2B, Equipar, Brazil), placed in cryotubes were frozen by immersion in liquid nitrogen, and transported in a cryogenic shipper to the laboratory where they were stored at −80 °C. The plasma biochemical constituents were measured using an automated biochemical analyzer (Humastar 200®, In Vitro Diagnóstica, Brazil) and specific reagents according to the manufacturer's instructions. The biochemical panel included alanine aminotransferase (ALT), albumin, aspartate aminotransferase (AST), calcium, creatine phosphokinase (CPK), glucose, globulin, triglycerides, total cholesterol, total protein, iron, phosphorus, magnesium, potassium, sodium, urea, uric acid.

2.6. Heavy metal analysis

Heavy metal analysis was performed according to Camacho et al. (2013). Briefly, a 1-mL fraction of plasma was used for heavy metals quantification. These samples were microwave-digested in 6 mL of nitric acid using a Multiwave 3000 (Anton Paar®) system. The metals and metalloids (As, Cd, Cu, Cr, Fe, Hg, Mn, Pb, Zn) were quantified using the High-Resolution Continuum Source Graphite Furnace Atomic Absorption Spectrometer (HR-CS GF AAS, Analytik Jena, Jena, Germany) and mercury quantification was carried out using an atomic fluorescence spectrometer Mercur Duo Plus (Analytik Jena, Jena, Germany). A calibration curve with a Multi-element Standard Solution 6 (Sigma-Aldrich®) and two blanks were run during each set of analyses to check the purity of the chemicals, and the blank reading was subtracted from all experimental readings. To test the precision and accuracy of the analytical method, National Research Council Canada (NCR - CNRC) certificated reference materials (TORT-3, DOLT-5, DORM-4) were analyzed. All the values of the reference materials were within certified limits. Recovery values were: As 93%; Cd 93%; Cr 88%; Cu 91%; Fe 89%; Hg 96%; Mn 87%; Pb 88%; and Zn 84%. The metal concentrations are expressed as μg/g of w.w.

2.7. Statistical analysis

Data were summarized for each parameter using means, standard deviation (SD), and range (minimum–maximum) values. To test data normality and equality of variances, the Kolmogorov-Smirnov test and Levene's test were used, respectively. To assess differences between areas (Coroa Vermelha and Santa Cruz) and between individuals (with or without fibropapillomatosis) parametric (Student *t*-test: t) or nonparametric (Mann-Whitney *U* test: U) analyses were performed, as appropriate.

Kruskal–Wallis, followed by Dunn's multiple comparison test was used to estimate the differences among epibiont loads (mild, moderate, and heavy) and ectoparasite count (mild, moderate, and heavy) with body condition score. The relationship between each blood parameter and curved carapace length (as an indicator of body size), BCI, heavy metals, and the presence of fibropapillomatosis tumors were evaluated using Pearson's or Spearman's correlation test, depending on whether both parameters exhibited a normal distribution. Statistical Package for Social Science (SPSS) 20.0 was used to analyze the data and results with a $p \leq 0.05$ were considered significant. The integration of biochemical and hematological parameters and bioaccumulation of metals was explored with Principal Component Analysis (PCA) using the package FactoMineR in R software.

3. Results

A total of 124 green sea turtles were captured within the study locations. Primary turtles, those caught and tagged for the first time in this study, accounted for the majority (96%) of all turtles. At Coroa Vermelha, only one among 69 turtles captured, was recaptured and at Santa Cruz, four out of 55, were recaptured.

3.1. Morphometrics and health assessment

Animals ranged between 28 and 59.3 cm CCL (mean size of 42.4 ± 0.07 cm) and weighed from 2.3 to 19.0 kg (mean of 8.5 ± 4.4 kg). According to Chaloupka and Limpus (2005) criteria, 58 green sea turtles were defined as an immature-new recruit (<40 cm CCL) and 66 were juveniles (40–65 cm CCL). The body condition index (BCI) ranged between 0.91 and 1.80 (mean of 1.32 ± 0.14). Only three (2.4%) turtles had BCI lower than 1 (emaciated, Norton and Wyneken, 2015) and they also had fibropapillomatosis. Visual evaluation of BC was consistent with the calculated BCI. Since turtles in poor body condition had lower BCI values than turtles with average body condition, which in turn had lower BCI than the turtles in good body condition. Visual health assessments data from each area are shown in Table 1.

During the study, 37 turtles presented fibropapillomatosis (FP) representing 29.8% of all turtles caught. Turtles in Santa Cruz had a higher occurrence of FP (53%) than in Coroa Vermelha (12%). Considering tumor count, animals from Santa Cruz had 14% mild, 55% moderate, and 31% severe tumor scores. At Coroa Vermelha none of the FP-affected individuals presented a severe tumor score, and mild and moderate accounted for 50% each. The BCI of FP-free individuals ranged from 1.0 to 1.8 (1.32 ± 0.13), and between 0.91 and 1.62 (1.33 ± 0.16) in FP-affected individuals. Turtles had tumors in the neck (34/37, 91.8%), eyes (25/37, 67.5%), anterior flippers (32/37, 86.4%), posterior flippers (27/37, 79.4%), plastron (11/37, 29.7%), carapace (8/37, 21.6%) tail (9/37, 24.3%) and cloacae

Table 1
Visual health assessments findings from juvenile green sea turtles captured in Coroa Vermelha ($n = 69$) and Santa Cruz ($n = 55$).

Parameter	Coroa Vermelha		Santa Cruz	
	n	%	*n*	%
Body condition				
Good	43	62.3	42	76.4
Average	23	33.3	12	21.8
Poor	3	4.4	1	1.8
Epibiont loads				
None	17	24.6	23	41.8
Mild	40	58.0	25	45.5
Moderate	12	17.4	5	9.1
Heavy	0	0	2	3.6
Ectoparasites				
None	67	97.1	26	47.2
Mild	2	2.9	15	27.3
Moderate	0	0	10	18.2
Heavy	0	0	4	7.3
Fibropapilloma score				
None	61	88.4	26	47.2
Mild	4	5.8	4	7.3
Moderate	4	5.8	16	29.1
Severe	0	0	9	16.4

(6/37, 16.2%%). No visible tumors were found inside the mouth or throat. Epibiont loads did not vary among turtles with different body conditions (Kruskal-Wallis $\chi2 = 5.129$, $p = 0.07$) or fibropapillomatosis presence ($U = 1409$, $p = 0.23$). At Coroa Vermelha 75% of green sea turtles had epibionts compared to 58% at Santa Cruz. However, there was no significant statistical difference between locations regarding this parameter ($U = 1574$, $p = 0.07$). Ectoparasites were significantly higher in turtles from Santa Cruz (29/55, 53%), than in turtles from Coroa Vermelha (2/69, 3%) ($U = 938$, $p < 0.001$). Ectoparasites count did not vary among body conditions (Kruskal-Wallis $\chi2 = 0.649$, $p = 0.72$), but sea turtles with fibropapillomatosis had significantly higher numbers of ectoparasites ($U = 954.5$, $p < 0.0001$) (Table 1).

3.2. Hematology

Results from hematology, blood biochemistry, and heavy metal analyses of *C. mydas* in the studied areas are shown in Table 2. In all cases, blood smear examinations were negative for hemoparasites. Animals from Santa Cruz showed significantly lower levels of hemoglobin ($t = 2.746$, $p = 0.007$), MCH ($U = 1442$, $p = 0.031$), MCHC ($U = 1447$, $p = 0.04$), and eosinophils ($U = 818$, $p < 0.0001$), and significantly higher levels of leukocytes ($U = 1420$, $p = 0.023$), thrombocytes ($U = 1265$, $p = 0.002$) and heterophils ($U = 1080$, $p < 0.0001$) than animals from Coroa Vermelha.

3.3. Plasma biochemistry

Biochemistry analyses showed elevated levels of urea ($U = 1173$, $p = 0.0002$), phosphorus ($t = 4.877$, $p < 0.0001$), sodium ($U = 1425$, $p = 0.016$), ALT ($U = 277.5$, $p = 0.025$) and AST ($U = 1357$, $p = 0.006$) in animals from Santa Cruz. On the contrary, lower levels of calcium ($t = 5.158$, $p < 0.0001$), glucose ($t = 3.106$, $p = 0.002$), total cholesterol ($U = 1168$, $p = 0.0002$), triglycerides ($U = 996$, $p < 0.0001$), total protein ($t = 3.506$, $p = 0.0006$), albumin ($t = 2.104$, $p = 0.037$), globulin ($t = 3.855$, $p = 0.0002$) and CPK ($U = 1498$, $p = 0.044$) were observed in green sea turtles from Santa Cruz compared to animals captured in Coroa Vermelha.

3.4. Heavy metal analysis

Trace element distribution in blood was Fe > Zn > Mn > Cu > As > Cr > Pb > Cd > Hg for animals sampled in both areas. Higher levels of arsenic ($U = 1423$, $p = 0.016$) and copper ($U = 1120$, $p = 0.0002$) were found in *C. mydas* from Santa Cruz, while turtles from Coroa Vermelha showed higher levels of iron ($U = 1187$, $p = 0.0003$) and mercury ($U = 834.5$, $p < 0.0001$). No strong correlations were found between heavy metals in blood and the turtle's size or weight. When metals co-variations were analyzed, significant positive correlations were observed between, As and Cu ($r = 0.598$, $p < 0.0001$), As and Zn ($r = 0.569$, $p < 0.0001$), as well as Cu and Zn ($r = 0.526$, $p < 0.0001$), and negative correlations between As and Cd concentrations ($r = -0.530$, $p < 0.0001$). Also, Cu concentrations positively correlated with urea ($r = 0.525$, $p < 0.0001$) and Cd concentrations negatively correlated with lymphocytes ($r = -0.503$, $p < 0.0001$).

Data related to each target metal in the blood and biochemical and hematological parameters were assessed by PCA analyses. The resulting PCA yielded two principal components that contained 38.1% of the total variance (26.5% for PC1 and 11.6% for PC2) (Fig. 2). The PCA analysis showed that health parameters and heavy metal levels were grouped accordingly to study sites with some level of overlap among them, separating the plotted results among the animals from Santa Cruz and animals from Coroa Vermelha. Parameters related to nutrition (protein, triglycerides, cholesterol, glucose) are mostly related to animals from Coroa Vermelha and external health indicators (fibropapilloma presence, ectoparasites) were mostly related to samples obtained at Santa Cruz.

4. Discussion

Five years after the collapse of Fundão dam, the impacts on the ecosystem are still uncertain. Few studies have been published describing the magnitude and reversion perspective of the effects on the habitat and the species affected (Bianchini et al., 2016; GIAIA, 2017; Queiroz et al., 2018; Bonecker et al., 2019; Costa et al., 2019). This study sought to analyze the health condition of *C. mydas* from a foraging area affected by the tailings and to compare the results with animals in a close foraging area that was not directly impacted. Studies like this are important to give a dimension of the environmental changes caused by mining tailings as to provide valuable information to forecast future changes, and thus, to plan effective mitigation measures and conservation strategies.

4.1. Hematology

Most of the turtles sampled at Santa Cruz (SC) and Coroa Vermelha (CV) were apparently healthy based on visual assessments. Juvenile green sea turtles from the most affected area (Santa Cruz) had significantly lower hemoglobin levels than turtles captured in Coroa Vermelha, which were reflected in erythrocytic indexes (MCH and MCHC). Mean Hb concentration (5 g/dL) of SC turtles is the lowest value ever reported for juvenile green sea turtles in Brazil (Marcon et al., 2015, Mello and Alvarez, 2019, Rossi et al., 2009, Santos et al., 2009, Zwarg et al., 2014), even if compared to turtles with fibropapillomatosis (Mello and Alvarez, 2019, Rossi et al., 2009, Zwarg et al., 2014) (Table 3). Although statistical analysis did not reveal a significant difference in hematocrit and red blood cell counts between SC and CV turtles, their mean values (17% and 0.31 $\times$ 10^6/μL, respectively) were also the lowest values found in Brazil (Marcon et al., 2015, Mello and Alvarez, 2019, Rossi et al., 2009, Santos et al., 2009, Zwarg et al., 2014), and Hct is below the normal ranges (Hct: 19% to 45%) for Brazilian juvenile green sea turtles (Santos et al., 2009, 2015).

The decrease of these parameters (Hb, Hct, RBC, MCH, and MCHC) define a framework of anemia. Anemias are usually caused by blood-sucking parasites or traumatic injuries as well as coagulopathy or the presence of ulcerative lesions. Other factors that could be associated to anemia in reptiles include iron deficiency, chronic renal or hepatic disease, chemicals, neoplasia, or possibly hypothyroidism (Campbell, 2006). The higher presence of ectoparasites in these animals (53%) may be contributing to anemia. In chelonians, RBC indices may be interpreted as a comparative index of nutrition, or general health (Campbell, 1998, 2006; Oliveira-Junior et al., 2009) because anemia is a frequent effect of chronically poor nutritional status, particularly concerning protein intake (Christopher, 1999).

Table 2
Morphometrics, hematology, plasma biochemical parameters, and heavy metals levels from juvenile green sea turtles captured in a mining tailing affected area at Santa Cruz and a not directly affected area at Coroa Vermelha.

Parameter	Coroa Vermelha					Santa Cruz				
	n	mean	SD	Min	Max	*n*	mean	SD	Min	Max
Biometrics										
Weight (kg)**	69	10.0	4.79	2.7	21.7	55	6.66	2.97	2.3	18.0
CCL (m)**	69	0.453	0.071	0.300	0.593	55	0.388	0.051	0.280	0.547
CCW (m)**	69	0.408	0.061	0.280	0.537	55	0.354	0.050	0.240	0.501
BCI	69	1.27	0.14	0.91	1.63	55	1.38	0.12	0.99	1.80
Hematology										
Red blood cell ($\times 10^6$/μL)	69	0.33	0.09	0.12	0.58	54	0.31	0.15	0.11	0.87
Hemoglobin (g/dL)*	69	6.05	1.98	2.00	10.8	54	5.00	2.24	1.20	9.40
Hematocrit (%)	69	20	6	6	40	54	17	8	6	32
MCV (fl)	69	623	180	242	1042	54	598	260	73	1532
MCH (pg)**	69	193	62	53	444	54	172	73	61	349
MCHC (g/dL)**	69	32.0	9.93	14.1	75.0	54	28.9	8.22	10.0	52.0
Leukocytes/μL**	69	5866	3556	1500	16,500	54	6813	2905	2500	14,250
Thrombocytes/μL**	69	2978	1833	375	8250	54	3616	1369	875	7500
Heterophils (%)**	68	41	14	13	87	54	52	17	9	92
Lymphocytes (%)	68	36	14	5	67	54	32	15	3	76
Monocytes (%)	68	12	10	0	52	54	10	7	0	26
Eosinophils (%)**	68	11	6	1	28	54	5	4	0	22
Basophils (%)	68	0	0	0	0	54	0	0	0	1
Heterophils/μL**	68	2267	1337	488	8051	54	3574	2160	371	13,110
Lymphocytes/μL	68	2123	1713	113	10,480	54	2178	1439	333	5719
Monocytes/μL	68	852	1249	0	8580	54	679	580	0	2231
Eosinophils/μL**	68	626	536	15	2543	54	366	351	0	1678
Basophils/μL**	68	0	0	0	0	54	2	13	0	94
Biochemistry										
Uric acid (mg/dL)	69	0.92	0.53	0.04	2.40	55	1.07	0.81	0.11	3.80
Urea (mg/dL)**	69	25.4	43.4	5.30	250	55	40.4	45.7	6.1	253
Calcium (mg/dL)*	69	7.26	2.26	2.30	12.3	55	5.41	1.55	2.3	8.6
Phosphorus (mg/dL)*	69	4.97	1.33	2.20	8.10	55	6.20	1.47	3.27	8.84
Sodium (mEq/L)**	69	144	5.26	134	171	55	145	4.84	134	155
Potassium (mEq/L)	69	4.25	0.54	3.23	6.20	55	4.13	0.65	3.00	6.50
Glucose (mg/dL)*	69	77.2	12.8	48.0	116	55	70.0	12.7	48.0	114
Total Cholesterol (mg/dL)**	69	96.7	56.5	12.0	304	55	64.9	41.6	11.0	230
Triglycerides (mg/dL)**	69	102	95.3	8.00	740	55	51.4	32.8	7.00	192
Total protein (g/dL)*	69	2.83	0.81	1.00	4.60	55	2.29	0.87	0.40	4.40
Albumin (g/dL)*	69	0.78	0.26	0.30	1.70	55	0.68	0.25	0.20	1.43
Globulin (g/dL)*	69	2.05	0.62	0.70	3.54	55	1.60	0.67	0.20	3.13
ALT (U/L)**	69	3.35	1.49	1.00	9.60	55	3.47	2.02	1.30	11.1
AST (U/L)**	69	112	61	30.9	428	55	132	59	42.1	360
Alkaline phosphatase (U/L)	69	16.1	8.36	1.40	39.0	55	14.1	8.24	3.20	38.6
Serum iron (μg/dL)	69	25.34	8.93	6.00	55.5	55	31.6	35.3	7.00	249
CPK (U/L)*	69	1155	2373	9.00	19,405	55	791	1288	8.00	8685
Magnesium (mg/dL)	69	7.23	1.85	4.31	11.4	55	6.79	1.88	1.80	11.9
Heavy metals										
As (μg/L)**	69	42.5	42.7	0.70	164	55	44.7	27.5	0.98	111
Cd (μg/L)	65	0.583	0.466	0.001	2.102	55	0.659	0.504	0.000	1.826
Cr (μg/L)	69	11.6	25.1	0.62	195	55	9.2	15.3	0.56	101
Cu (μg/L)**	69	63.4	100	0.26	427	55	90.6	86.3	5.91	433
Fe (μg/L)**	69	1593	1237	173	5760	55	1146	1404	45.5	7212
Hg (μg/L)**	69	0.21	0.17	0.01	0.99	55	0.11	0.09	0.02	0.59
Mn (μg/L)	69	116	18.8	89.3	183	55	112	46.9	31.9	382
Pb (μg/L)	68	4.20	4.41	0.00	24.09	55	3.80	3.31	0.00	13.27
Zn (μg/L)	69	364	268	0.86	1118	55	610	857	11.0	4631

CCL curved carapace lengh, *CCW* curved carapace width, *BCI* body condition index, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CPK* creatine phosphokinase, *As* arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Pb* lead, *Zn* zinc.

* Significant difference by Student *t*-test ($p < 0.05$).

** Significant difference by Mann-Whitney *U* test ($p < 0.05$).

When comparing the white blood cell values from turtles of the two sampled areas, SC turtles presented higher WBC, thrombocytes and heterophils count, and lower eosinophils count (Table 2). Elevated total WBC may reflect inflammation and active immune responses, infection, and/or stress in reptiles (Sykes and Klaphake, 2015). Heterophils are primarily phagocytic, thus related with disorders and conditions that are characterized by inflammation, particularly those associated with infection or tissue injury (Campbell, 2006). Eosinophils are also phagocytic and especially involved in parasites destruction. In chelonians, eosinophils participate in the immune response and phagocytize immune complexes (Zhang et al., 2011). The leukocytosis and heterophilia observed in turtles most affected by the tailings could be associated with chronic and active infectious processes (Sykes and Klaphake, 2015), since they have a higher prevalence of fibropapillomatosis, whereas the significance of eosinopenia is unknown in reptiles (Irizarry-Rovira, 2004). Overall, these clinical data indicate that sea turtles at Santa Cruz present a physiological deficit in oxygen

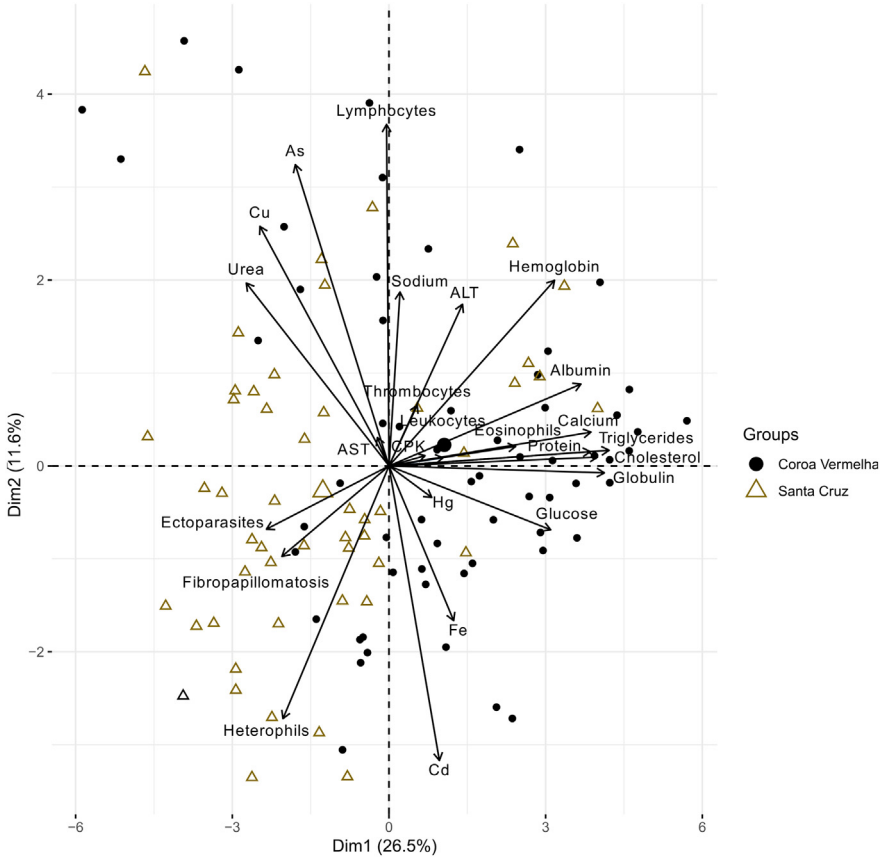

Fig. 2. Principal Component Analysis (PCA) plot showing the multivariate variation between heavy metals and biochemical and hematological parameters. Vectors indicate the direction and strength of each variable to the overall distribution. Black dots correspond to Coroa Vermelha turtles and brown open triangles correspond to Santa Cruz turtles.

transport by red blood cells caused by the lower levels of hemoglobin. The heterophilia and leukocytosis observed could be attributed to greater microbial or inflammatory challenges (Villa et al., 2017) and the thrombocytosis may appear in response to tumors (like fibropapillomas), chronic inflammation, bleeding, or iron deficiency (Theml et al., 2004).

4.2. Plasma biochemistry: Protein homeostasis

Juvenile green sea turtles from the most affected area also had significantly lower albumin, globulin, and protein levels, when compared with green sea turtles captured in Coroa Vermelha. In reptiles, albumin is the most important factor for the maintenance of the blood oncotic pressure and can reflect changes in the nutritional status and health condition, or adaptations to the habitat (Masat and Dessauer, 1968; Goldwasser and Feldman, 1997; Whiting et al., 2007). Albumin concentration may be helpful as a prognostic indicator in diseased animals since during chronic and acute inflammation, the liver produces large amounts of mediators, and albumin is not a reactant, so its synthesis may reduce (Doweiko and Nompleggi, 1991). Hypoproteinemia has also been demonstrated in animals that are in poor health conditions (Flint et al., 2009) like turtles severely affected by fibropapillomatosis (Aguirre and Balazs, 2000), or in debilitated animals like stranded loggerhead turtles found along the Georgia coast (Deem et al., 2009). The authors attributed the low blood protein levels to a food intake decrease, malnutrition, parasitism, renal diseases, or a protein assimilation decrease. Diseased loggerhead and green sea turtles that were captured at an intake canal of a nuclear power plant also showed decreased concentrations of total protein, albumin, α-globulin, and β-globulin and A:G ratio (Osborne et al., 2010). Therefore, the lower values of albumin and protein observed in animals from SC may reflect lower levels of dietary protein intake and at least some influence of chronic inflammation that could be related to fibropapillomatosis (Flint et al., 2009; Whiting et al., 2007).

Additionally, the significant reduction in total protein along with lower globulin levels in SC turtles may be reflecting a decrease in antibody synthesis. Perrault et al. (2017) noticed a significant negative correlation between γ-globulins and blood arsenic, suggesting an alteration of humoral immunity. Arsenic has also been associated with reduced albumin and WBC count in loggerhead sea turtles (Register, 2011; Camacho et al., 2013) and reduced total globulins in green sea turtles (Komoroske et al., 2011). In this study, it was not found any strong correlation between arsenic and blood parameters, however, SC turtles had significantly higher arsenic levels and lower protein, albumin, and globulin levels compared to CV turtles. Arsenic contamination may prevent the immune system from fighting off microorganisms, predisposing to infection (Brown et al., 1999). The higher incidence of fibropapillomatosis in the most affected area is another evidence of immunosuppression (Work et al., 2001). The elevated arsenic plasma concentrations observed in SC turtles suggest a recent exposure to this metal, that is abundant in the region (Mirlean et al., 2013). Food intake is a major contamination route in sea turtles (Agusa et al., 2008) and according to Van de Merwe et al. (2010) blood arsenic levels can reflect tissue levels of this contaminant.

Studies show that cadmium has also a potential immunosuppressive effect on the immune system of sea turtles. In nesting loggerhead turtles from Florida, cadmium was responsible for a reduction in total globulins (Perrault et al., 2017). In green sea turtles from San Diego Bay, blood cadmium levels negatively correlated with total globulins (Komoroske et al., 2011). In the present study, a negative correlation was found between cadmium levels and lymphocytes, which can also be linked to a suppression of antibody production.

Table 3
Mean values (min − max) of hematological parameters for green sea turtles with or without fibropapillomatosis from different areas in Brazil.

Study area (*n*)	CCL (cm)	FP	RBC ($\times 10^6$/μL)	Hb (g/dL)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	LEU/μL	THROMB/μL	HET/μL	LYM/μL	MO/μL	EO/μL	BA/μL	Reference
Coroa Vermelha (69)	45.3	–	0.33 (0.12–0.58)	6.05 (2.0–10.8)	20 (6–40)	623 (242–1042)	193 (53–444)	32 (14.1–75)	5866 (1500–16,500)	2978 (375–8250)	2267 (488–8051)	2123 (113–10,480)	852 (0–8580)	626 (15–2543)	0 (0–0)	This study
Santa Cruz (55)	38.8	–	0.31 (0.11–0.87)	5 (1.2–9.4)	17 (6–32)	598 (73–1532)	172 (61–349)	28.9 (10.0–52.0)	6813 (2500–14,250)	3616 (875–7500)	3574 (371–13,110)	2178 (333–5719)	679 (0 – 2231)	366 (0–1678)	2 (0–94)	This study
Angra dos Reis (33)	39.7	No	0.49 (0.25–0.9)	10.13 (7.3–16.3)	30.4 (22–49)	667 (386–1088)	–	34.2 (33–37)	3782 (1000–9000)	–	647.3 (24–4160)	98.61 (0–960)	258 (0–3360)	199 (0–2560)	13 (0 – 320)	Marcon et al. (2015)
Fernando de Noronha (60)	55.6	No	0.39 (0.24–0.55)	10 (5.9–14)	29 (21.4–36.6)	743 (500–986)	255 (144–367)	34.4 (23.7–45.1)	3553 (1178–8259)	20,535 (9513–36,316)	1926 (621–4317)	712 (221–1924)	333 (15.4–1494)	575 (96.1–1831)	4.8 (0–45.3)	Santos et al. (2009)
Lagamar estuary lagoon (39)	34.0	No	0.47 (0.3–0.64)	9.0 (5.9–11.1)	34 (23–43)	720 (500–1000)	190 (140–260)	26.3 (22.2–31.3)	3670 (1000–8000)	6430 (2000–16,000)	2761 (650–5920)	755 (210–2080)	29 (0 – 210)	46 (0–280)	6.79 (0–60)	Mello and Alvarez (2019)
Lagamar estuary lagoon (9)	41.4	Yes	0.35 (0.18–0.5)	7.3 (6.4–9.7)	23 (15–32)	710 (500–1000)	170 (90–250)	25.6 (20.7–30.6)	3020 (1000–8000)	9220 (3000–16,000)	2090 (790–6160)	865 (120–1850)	49 (0–240)	11 (0–40)	4.44 (0–40)	Mello and Alvarez (2019)
Ubatuba (18)	37.9	No	0.39	8.15	27.6	718	234	29.72	6590	14,870	5128	789	339	105	0	Zward et al. (2014)*
Ubatuba (29)	44.4	Yes	0.44	6.88	24.3	608	171	28.39	6380	15,820	4996	938	424	211	0	Zward et al. (2014)*
Ubatuba (18)	36.4	No	0.36	7.11	24.6	618	217	29.41	5545	16,500	4316	799	258	171	0	Rossi et al. (2009)*
Ubatuba (12)	44.2	Yes	0.43	6.41	22.7	626	174	28.5	7125	16,160	4266	974	360	123	0	Rossi et al. (2009)*

CCL curved carapace length, *FP* fibropapillomatosis, *RBC* red blood cells, *Hb* hemoglobin, *Hct* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *LEU* leukocytes, *THROMB* thrombocytes, *HET* heterophiles, *LYM* lymphocytes, *MO* monocytes, *EO* eosinophils, *BA* basophils.
* Results presented only as mean and standard deviation.

4.3. Plasma biochemistry: Ions

Differences in ions concentrations were also found between areas. SC turtles presented significantly lower levels of calcium and higher phosphorus levels. The calcium/phosphorus ratio (P:Ca) is commonly used in reptile medicine to evaluate mineral balance with respect to renal and nutritional status (Klaphake, 2010). This ratio is modified at an initial stage of renal disease in comparison to uric acid (Kölle and Hoffmann, 2001). P and Ca are macronutrients of high importance in the organism. They are components of the bone matrix, and the imbalance of one of them leads to an increase or decrease in the other, which can culminate in some bone metabolic diseases, like osteodystrophy and retarded growth (Fowler, 1986). Elevated serum levels of phosphorus and alterations in the P:Ca ratio may occur in renal insufficiency (Kölle and Hoffmann, 2001). The difference in plasma phosphorus and calcium concentrations between study sites could be explained by the different degrees of eutrophication related to the distance from the coast, resulting in different levels of phosphorus in food components or the beginning of chronic renal disease.

4.4. Plasma biochemistry: Enzymes

When comparing enzyme values from turtles of the two sampled areas, SC turtles presented higher AST and ALT, and lower CPK levels. Aspartate aminotransferase (AST) is found in greater concentrations in both muscle cells and hepatocytes, with a wide distribution throughout the reptile body (Thrall, 2006). Consequently, high plasma AST levels alone are not considered indicative of liver disease (Anderson et al., 2013). However, when no elevations are observed in creatine phosphokinase (CPK) levels, a hepatocellular damage is probable (Campbell, 2015) because CPK is an indicator of muscle lesions (Kramer and Hoffmann, 1997). In this scenario, the significant elevation in AST and alanine aminotransferase (ALT) activities along with a reduction in CPK levels in SC turtles is most likely of a liver rather than muscle origin and may suggest that these turtles are in the early stages of liver disease development.

The greater levels of arsenic and copper found in SC turtles may also be responsible for the elevation of AST and urea levels. Labrada-Martagón et al. (2010) noticed higher concentrations of AST and ALT in green sea turtles and attributed these elevations to a physiological response to some contaminants and hepatocellular damage. Ley-Quiñónez et al. (2017) found significant positive correlations between ALP and arsenic in Mexican loggerhead turtles and argumented that the increases in ALP activity may be caused by arsenic accumulation. Cortés-Gómez et al. (2018a) also observed positive relationships between arsenic and AST and arsenic and urea in nesting olive ridley sea turtles from Mexico. In humans, higher copper levels are related to chronic renal disease (Mafra, 2003). As already seen, AST can be a biomarker for liver function and in turn, urea can be used to estimate renal function and glomerular filtration. Thus, the changes observed in AST, ALT, CPK, and urea levels are suggestive of hepatic, renal, and/or systemic diseases in SC turtles (Mader, 2005, Flint et al., 2009, Anderson et al., 2011, 2013).

4.5. Heavy metals

Trace element distribution in blood had similar profiles in samples from both study areas (Fe > Zn > Mn > Cu > As > Cr > Pb > Cd > Hg), which was expected as essential metals are involved in several important physiological and homeostatic processes (Bury et al., 2003), while non-essential metals are not actively controlled by sea turtles and could change according to the exposure level (Maffucci et al., 2005). SC turtles showed higher As and Cu levels than CV turtles. Comparing with other green sea turtles' populations in Brazil, the levels of Cu, Mn, Zn, As, Cd and Pb in SC turtles were higher than turtles from Fernando de Noronha (Prioste et al., 2015) (Table 4). Concentrations of Cu, Zn, Cd, and Pb were also higher in SC turtles than green sea turtles captured in Ubatuba (Silva et al., 2016). *C. mydas* sampled in Vitória had the highest levels of all metals analyzed (Prioste,

Table 4
Metal levels (mean ± SD, ug g^{-1} wet weight) in blood of green sea turtles at different areas in Brazil.

Study areas	Location information	*n*	CCL	Method	Essential metals					Non-essential metals				Source
					Cr	Cu	Fe	Mn	Zn	As	Cd	Hg	Pb	
Almofala - CE	Village inhabited by indigenous people	24	48.4	ICP-MS	–	–	–	–	7.58 ± 4.17	0.93 ± 0.90	0.016 ± 0.016	0.009 ± 0.008	0.029 ± 0.027	Prioste (2016)
Vitória - ES	Final effluent of a steel mill	68	42.8	ICP-MS	–	–	–	–	21.53 ± 46.08	2.16 ± 5.15	0.009 ± 0.011	0.010 ± 0.017	1.12 ± 2.04	Prioste (2016)
Fernando de Noronha - PE	Oceanic island without industrial development and with low environmental impacts	31	72.0	ICP-MS	–	0.75	–	0.61	0.14	0.2	0.01	–	0.02	Prioste et al. (2015)
		71	68.7	ICP-MS	–	–	–	–	8.31 ± 4.26	5.04 ± 39.7	0.010 ± 0.009	0.0002 ± 0.0002	0.027 ± 0.020	Prioste (2016)
Ubatuba - SP	Foraging area with urban development and waste discharge	70	39.3	ICP-MS	–	–	–	–	6.94 ± 5.79	2.17 ± 3.97	0.012 ± 0.013	0.022 ± 0.037	0.034 ± 0.029	Prioste (2016)
		13	37.0	AAS	–	0.92	–	–	0.68	–	0.078	–	0.954	Silva et al. (2016)*
Santa Cruz - ES	Estuarine that receives waste from urban and industrial discharged and was affected by mining tailings	55	38.8	GFASS	0.09 ± 0.15	0.90 ± 0.86	11.46 ± 14	1.12 ± 0.4	6.10 ± 8.5	0.44 ± 0.27	0.006 ± 0.005	0.001 ± 0.0009	0.038 ± 0.03	Present study
Coroa Vermelha - BA	Foraging ground distant from anthropogenic disturbances	69	45.3	GFASS	0.11 ± 0.25	0.63 ± 1.0	15.93 ± 12	1.16 ± 0.1	3.64 ± 2.6	0.42 ± 0.42	0.005 ± 0.004	0.002 ± 0.001	0.042 ± 0.04	Present study

CCL curved carapace length, *As* arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Pb* lead, *Zn* zinc, *AAS* atomic absorption spectrophotometry, *ICP-MS* inductively coupled plasma- mass spectrometry, *GFAAS* - graphite furnace atomic absorption spectroscopy.

* Results originally published in dry weight transformed into wet weight using the humidity percentage reported by Guirlet et al., 2008 (Blood, 80%).

2016). These animals were captured in the final effluent of a steel mill, therefore, an inadequate place to serve as a reference.

CV turtles had higher blood Hg levels than SC turtles, however, the concentrations found in this study are low comparing with other populations (Prioste, 2016; Silva et al., 2016) and they do not seem to be affecting the health of these turtles.

4.6. Data integration

Green turtles from the indirectly affected area (CV) demonstrated a good body condition, which coincided with higher concentrations of glucose, lipids (cholesterol and triglycerides), total proteins, albumin, and lower activity of ALT and AST than SC turtles. The PCA analysis exhibited in Fig. 2 demonstrates that this statement is true since levels of albumin, calcium, triglycerides, total cholesterol, glucose, total protein were mostly related to samples obtained at Coroa Vermelha. These parameters may reflect a food-enriched environment, with greater food availability and/or better food quality (Labrada-Martagón et al., 2010). Green sea turtles' diet relies on the food sources availability and the selection made by each sea turtle (López-Mendilaharsu et al., 2005). The diet's nutritional content is determined by the capacity of the individual to assimilate the nutrients (Bjorndal, 1985) and the chemical composition of the food consumed (Villegas-Nava, 2006).

Santos et al. (2011) and Bastos (2018) evaluated the impact of coastal habitat degradation on the food availability for green sea turtles, and the chemical composition and the nutritional value of the diet, respectively. The area they studied is Vitória, a highly urbanized site with wastewater discharge from industries and residences, about 50 km south of Santa Cruz. Santos et al. (2011) found that environmental degradation is causing a decrease in species diversity in the study area with a predominance of opportunistic algae and dominance of *Caulerpa mexicana*. This low number of available food items, along with the possible secondary metabolite production by *Caulerpa mexicana*, may force green sea turtles to base their diet mostly in one genus of marine algae (*Ulva* sp.), resulting in a little diverse and restricted diet. Bastos (2018) observed that green sea turtles from Vitória had a high-calorie diet, which does not guarantee a balanced food intake due to the reduced availability of items. The loss of chemical diversity in the region and the reduction of available algae species influenced the nutritional balance of turtles. McDermid et al. (2007) noticed that Hawaiian green turtles tend to feed on seagrasses, marine algae, and algal turfs, which have considerable variation in nutritional composition. To optimize different life cycle processes such as survival, growth, and fecundity, and to help in the proper functioning and response to diseases, like fibropapillomatosis, a diversity in food items is required, thus sea turtles with restricted diets may be adversely affected (Worm et al., 2006).

Triglyceride levels are a useful tool for nutritional status assessment and general body condition of sea turtles (Aguirre and Balazs, 2000; Swimmer, 2000; Hamann et al., 2005). It has been proposed that, together with other metabolites like total protein, glucose, and cholesterol, the assessments of triglycerides should be incorporated and used in research about sea turtles' nutrition (Hamann et al., 2005; Whiting et al., 2007). The lower glucose, cholesterol, triglycerides, total protein, and calcium levels, and higher levels of sodium, phosphorus, urea, AST, and ALT in SC turtles reflect a worse nutritional state. If the food from Santa Cruz represents a suboptimal diet, a decrease in BC would also be expected in sea turtles that feed in that area, but it was not the case. Bastos (2018) observed that FP-turtles from Vitória (30 km southward from Santa Cruz) had good BCI, which is a result of a high-calorie diet in this region despite the low algae chemical diversity and the reduced availability of items. It seems that the differences in the BCI and biochemical parameters found between SC and CV areas reflect changes in habitat, availability, and composition of food.

Although no reduction in BCI in SC turtles was observed, the majority of anomalous biochemical findings (lower levels of glucose, cholesterol, triglycerides, total protein, and calcium, and higher levels of sodium, phosphorus, urea, AST, and ALT) reflect a worse nutritional state, hepatic tissue lesion, and possible renal disease. This scenario indicates that an

important subclinical physiological deficit may occur, and it can affect the immune system and animal behavior, which is corroborated by the higher ectoparasitic load and the greater incidence of fibropapillomatosis, as PCA analysis demonstrates (Fig. 2).

It is generally accepted that greater epibiont load on sea turtles is indicative of poor health (Deem et al., 2009). In the present study, it was observed that turtles from the indirectly affected area had higher epibiotic loads. However, the turtles had a good body condition, normal biochemical and hematological patterns and epibiont loads were not correlated with health parameters. In small immature turtles, Stamper et al. (2005) observed that the carapace barnacle burdens were not associated with health status, consistent with our findings. Excessive epibiont load is probably a consequence of the turtle's inability to access cleaning stations to control them (Losey et al., 1994). On the other hand, the lower epibiotic load found in SC turtles may be related to a less favorable habitat, causing the death of the less tolerant epibionts (Frick and Pfaller, 2013).

In summary, the variations in blood metal levels observed in this study can be explained by the recent exposure at the feeding area revealing different metal signatures between CV and the directly affected area (SC). Seagrasses are known as metal accumulators and can sequester them from the sediment in which they grow and the water (Besar et al., 2008). They represent an important dietary exposure source to sea turtles (Komoroske et al., 2012). It is thus possible that sea turtles are subjected to higher contaminant exposure via these events and pathways. The suspected anemia, immunosuppression, and hepato-renal pathologies seen in juvenile green sea turtles from the most affected area, may be a combination of their nutritional status, deteriorating health along with the associated effects on contaminant mobilization, detoxification, and elimination pathways. However, more studies are needed to analyze the macroalgae of SC and CV, regarding its bioaccumulation of heavy metals, and its biochemical diversity and community structure to confirm if it is causing the nutrient-deficient states and declining health of SC sea turtles. Additionally, interactions among metals could be related to synergistic or antagonistic effects that can decrease or increase the physiological or pathological consequences of each metal, accelerating its elimination or accumulation. Therefore, more studies are necessary to assess the health of sea turtles over time and ontogenetically, since the high prevalence of fibropapillomatosis and ectoparasites combined with a potential nutritional deficit, and the continuous and increasing degradation of the coastal areas, create a high-risk scenario for the species.

5. Conclusions

This study provides important information about juvenile green sea turtles' health that feed in an area directly affected by the tailings of a collapsed mining dam in Brazil. Santa Cruz presents a complex situation regarding the health of green turtles, as they are less healthy, with a higher prevalence of fibropapillomatosis and ectoparasites, and presented elevated levels of metals (As and Cu) compared to the not directly affected area Coroa Vermelha. These metal levels are also greater than other areas in Brazil that are less subject to coastal pollution, such as Fernando de Noronha Island (Prioste et al., 2015), even considering their particularities and the multifactorial influence on health. This situation requires long-term monitoring to quantify the direct or indirect influence of metal levels on the health of these juvenile green turtles. Understanding the metals' mechanisms of action allows an accurate risk assessment, which can help to predict and prevent wildlife damage, and is essential in guiding regulatory decisions for national conservation plans development. The continuous monitoring of pollutants coupled with physiological and immunological effects studies will increase our knowledge about contaminant impacts on sea turtles inhabiting polluted coastal areas.

CRediT authorship contribution statement

Camila Miguel: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Writing - Original Draft; Visualization **Patrícia Gomes Costa**: Investigation; Validation **Adalto Bianchini**: Methodology; Resources; Writing - review & editing **Octavio Luis Pérez Luzardo**: Conceptualization; Writing - review & editing **Monica Ryff Moreira Vianna**: Conceptualization; Writing - review & editing **Marcelo Renan de Deus Santos**: Conceptualization; Funding acquisition; Investigation; Methodology; Resources; Supervision; Writing - review & editing.

Declaration of competing interest

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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References

Aguirre, A.A., Balazs, G.H., 2000. Blood biochemistry values of green turtles, Chelonia mydas, with and without fibropapillomatosis. Comp. Haematol. Int. 10, 132–137. https://doi.org/10.1007/s005800070004.

Agusa, T., Takagi, K., Kubota, R., Anan, Y., Iwata, H., Tanabe, S., 2008. Specific accumulation of arsenic compounds in green turtles (Chelonia mydas) and hawksbill turtles (Eretmochelys imbricata) from Ishigaki Island, Japan. Environ. Pollut. 153, 127–136. https://doi.org/10.1016/j.envpol.2007.07.013.

Álvarez-Varas, R., Contardo, J., Heidemeyer, M., Forero-Rozo, L., Brito, B., Cortés, V., Brain, M.J., Pereira, S., Vianna, J.A., 2017. Ecology, health and genetic characterization of the southernmost green turtle (Chelonia mydas) aggregation in the eastern Pacific: implications for local conservation strategies. Lat. Am. J. Aquat. Res. 45, 540–554. https://doi.org/10.3856/vol45-issue3-fulltext-4.

Anan, Y., Kunito, T., Watanabe, I., Sakai, H., Tanabe, S., 2001. Trace element accumulation in hawksbill turtles (Eretmochelys imbricata) and green turtles (Chelonia mydas) from Yaeyama Islands, Japan. Environ. Toxicol. Chem. 20, 2802–2814. https://doi.org/10.1002/etc.5620201220.

Anderson, E.T., Minter, L.J., Clarke, E.O., Mroch, R.M., Beasley, J.F., Harms, C.A., 2011. The effects of feeding on hematological and plasma biochemical profiles in green (Chelonia mydas) and Kemp's Ridley (Lepidochelys kempii) sea turtles. Vet. Med. Int. 2011, 890829. https://doi.org/10.4061/2011/890829.

Anderson, E.T., Sacha, V.L., Gardner, M., Byrd, J.L., Manire, C.A., 2013. Tissue enzyme activities in the loggerhead sea turtle (Caretta caretta). J. Zoo. Wildl. Med. 44, 62–69. https://doi.org/10.1638/1042-7260-44.1.62.

Bastos, K.V., 2018. Perfil nutricional e análise comparativa dos recursos alimentares da tartaruga verde, Chelonia mydas, e a influência da urbanização em sua dieta. Master's ThesisUniversidade Federal do Espírito Santo 54 p.

Besar, S.N.T., Shazili, N.A.M., Abdullah, S.A., Mamat, A.S., 2008. Experimental and field study on accumulation of heavy metals in seagrasses (Halodule pinifolia and Halophila minor) in setiu wetland, Terengganu. J. Sustain. Sci. Manag. 3, 41–73.

Bianchini, A., Silva, C.C., Lauer, M.M., Jorge, M.B., Costa, P.G., Marques, J.A., Da-Silva, J.F., 2016. Avaliação do impacto da lama/pluma Samarco sobre os ambientes costeiros e marinhos (ES e BA) com ênfase nas Unidades de Conservação. 1a Expedição do navio de pesquisa Soloncy Moura do CEPSUL/ICMBio. Ministério do Meio Ambiente. Instituto Chico Mendes de Conservação da Biodiversidade–Icmbio. Diretoria de Pesquisa, Avaliação e Monitoramento da Biodiversidade, 62p. Available in: https://www.icmbio.gov.br/portal/images/stories/DCOM_relatorio_revisado_atualizado_29_04_2016_AB.pdf.

Bjorndal, K.A., 1985. Nutritional ecology of sea turtles. Copeia 1985, 736–751. https://doi.org/10.2307/1444767.

Bjorndal, K.A., 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, P.L., Musick, J.A. (Eds.), The Biology of Sea Turtles. CRC Press, Boca Raton, FL, pp. 199–231.

Bjorndal, K.A., Bolten, A.B., Chaloupka, M.Y., 2000. Green turtle somatic growth model: evidence for density dependence. Ecol. Appl. 10, 269–282. https://doi.org/10.1890/1051-0761(2000)010[0269:GTSGME]2.0.CO;2.

Bolten, A.B., 1999. Techniques for measuring sea turtles. In: Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., Donnelly, M. (Eds.), Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4, Washington, DC, pp. 110–114.

Bonecker, A.C.T., Castro, M.S., Costa, P.G., Bianchini, A., Bonecker, S.L.C., 2019. Larval fish assemblages of the coastal area affected by the tailings of the collapsed dam in Southeast Brazil. Reg. Stud. Mar. Sci. 100848. https://doi.org/10.1016/j.rsma.2019.100848.

Brown, M.B., McLaughlin, G.S., Klein, P.A., Crenshaw, B.C., Schumacher, I.M., Brown, D.R., Jacobson, E.R., 1999. Upper respiratory tract disease in the gopher tortoise is caused by mycoplasma agassizii. J. Clin. Microbiol. 37, 2262–2269. https://doi.org/10.1128/JCM.37.7.2262-2269.1999.

Bury, N.R., Walker, P.A., Glover, C.N., 2003. Nutritive metal uptake in teleost fish. J. Exp. Biol. 206 (1), 11–23. https://doi.org/10.1242/jeb.00068.

Camacho, M., Orós, J., Boada, L., Zaccaroni, A., Silvi, M., Formigaro, C., López, P., Zumbado, M., Luzardo, O., 2013. Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (Caretta caretta): results from a nesting colony from Cape Verde, West Africa. Mar. Environ. Res. 92, 15–22. https://doi.org/10.1016/j.marenvres.2013.08.002.

Campbell, T.W., 1998. Interpretation of the reptilian blood profile. Exotic Pet Pract. 3, 33–37.

Campbell, T.W., 2006. Clinical pathology of reptiles. In: Mader, D. (Ed.), Reptile Medicine and Surgery. Saunders Elsevier, St Louis, MO, pp. 453–470.

Campbell, T.W., 2015. Hematologia dos Répteis. In: Thrall, M.A., Weiser, G., Alisson, R., Campbell, T.W. (Eds.), Hematologia e Bioquímica Clínica Veterinária. Roca, São Paulo, pp. 239–250.

Carmo, F.F., Kamino, L.H.Y.K., et al., 2017. Fundão tailings dam failures: the environment tragedy of the largest technological disaster of Brazilian mining in global context. Perspect. Ecol. Conserv. 15, 145–151. https://doi.org/10.1016/j.pecon.2017.06.002.

Chaloupka, M., 2004. Exploring the metapopulation dynamics of the southern great barrier reef green sea turtle stock and the possible consequences of sex-biased local harvesting. In: Akcakaya, H., Burgman, M., Kindvall, O., Wood, C., Sjogren-Gulve, P., Hattfield, J., McCarthy, M. (Eds.), Species Conservation and Management: Case Studies. Oxford University Press, New York, pp. 340–354.

Chaloupka, M., Limpus, C., 2005. Estimates of sex- and age-class-specific survival probabilities for a southern great barrier reef green sea turtle population. Mar. Biol. 146, 1251–1261. https://doi.org/10.1007/s00227-004-1512-6.

Christopher, M.M., 1999. Physical and biochemical abnormalities associated with prolonged entrapment in a desert tortoise. J. Wildl. Dis. 35, 361–366. https://doi.org/10.7589/0090-3558-35.2.361.

Cortés-Gómez, A.A., Tvarijonaviciute, A., Teles, M., Cuenca, R., Fuentes-Mascorro, G., Romero, D., 2017. P-nitrophenyl acetate esterase activity and cortisol as biomarkers of metal pollution in blood of olive ridley turtles (Lepidochelys olivacea). Arch. Environ. Contam. Toxicol. 75, 25–36. https://doi.org/10.1007/s00244-017-0464-z.

Cortés-Gómez, A.A., Tvarijonaviciute, A., Girondot, M., Tecles, F., Romero, D., 2018a. Relationship between plasma biochemistry values and metal concentrations in nesting olive ridley sea turtles. Environ. Sci. Pollut. Res. Int. 25, 36671–36679. https://doi.org/10.1007/s11356-018-3467-z.

Cortés-Gómez, A.A., Morcillo, P., Guardiola, F.A., Espinosa, C., Esteban, M.A., Cuesta, A., Girondot, M., Romero, D., 2018b. Molecular oxidative stress markers in olive ridley turtles (Lepidochelys olivacea) and their relation to metal concentrations in wild populations. Environ. Pollut. 233, 156–167. https://doi.org/10.1016/j.envpol.2017.10.046.

Cortés-Gómez, A.A., Romero, D., Girondot, M., 2018c. Carapace asymmetry: a possible biomarker for metal accumulation in adult olive ridleys marine turtles? Mar. Pollut. Bull. 129, 92–101. https://doi.org/10.1016/j.marpolbul.2018.02.020.

Costa, G.B., Ramlov, F., Ramos, B., Koerich, G., Gouvea, L., Costa, P.G., Bianchini, A., Maraschin, M., Horta, P.A., 2019. Physiological damages of Sargassum cymosum and hypnea pseudomusciformis exposed to trace metals from mining tailing. Environ. Sci. Pollut. Res. 26, 36486–36498. https://doi.org/10.1007/s11356-019-06691-w.

Day, R.D., Segars, A.L., Arendt, M.D., Lee, A.M., Peden-Adams, M.M., 2007. Relationship of blood mercury levels to health parameters in the loggerhead sea turtle (Caretta caretta). Environ. Health Perspect. 115, 1421–1428. https://doi.org/10.1289/ehp.9918.

Deem, S.L., Norton, T.M., Mitchell, M., Segars, A.L., et al., 2009. Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (Caretta caretta) along the coast of Georgia, USA. J. Wildl. Dis. 45, 41–56. https://doi.org/10.7589/0090-3558-45.1.41.

Doweiko, J.P., Nompleggi, D.J., 1991. The role of albumin in human physiology and pathophysiology, part III: albumin and disease state. J. Parenter. Enter. Nutr. 15, 476–483. https://doi.org/10.1177/0148607191015004476.

Escobar, H., 2015. Mud tsunami wreaks ecological havoc in Brazil. Science 350, 1138–1139. https://doi.org/10.1126/science.350.6265.1138.

Fernandes, G.W., Goulart, F.F., Ranieri, B.D., 2016. Deep into the mud: ecological and socio-economic impacts of the dam breach in Mariana, Brazil. Nat. Conserv. 14, 35–45. https://doi.org/10.1016/j.ncon.2016.10.003.

Flint, M., Morton, J.M., Limpus, C.J., Patterson-Kane, J.C., Murray, P.J., Mills, P.C., 2009. Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (Chelonia mydas). Vet. J. 185, 299–304. https://doi.org/10.1016/j.tvjl.2009.06.011.

Francini-Filho, R.B., Cordeiro, M.C., Omachi, C.Y., Rocha, A.M., Bahiense, L., Garcia, G.D., Tschoeke, D., de Almeida, M.G., Rangel, T.P., De Oliveira, B.C.V., de Almeida, D.Q.R., Menezes, R., Mazzei, E.F., Joyeux, J.C., Rezende, C.E., Thompson, C.C., Thompson, F.L., 2019. Remote sensing, isotopic composition and metagenomics analyses revealed Doce River ore plume reached the southern Abrolhos Bank reefs. Sci. Total Environ. 697, 134038. https://doi.org/10.1016/j.scitotenv.2019.134038.

Frick, M.G., Pfaller, J.B., 2013. Sea turtle epibiosis. In: Wyneken, J., Lohmann, K.J., Musick, J.A. (Eds.), The Biology of Sea Turtles. III, pp. 399–426.

Fowler, M.E., 1986. Metabolic bone disease. In: Fowler, M.E. (Ed.), Zoo and Wild Animal Medicine. WB Saunders, Philadelphia, pp. 69–90.

Gastão, F.G.C., Silva, L.T., Lima Junior, S.B., Fernandes, L.F.L., Leal, C.A., Gobira, A.B., Maia, L.P., 2020. Marine habitats in conservation units on the southeast coast of Brazil. Braz. J. Dev. 6, 22145–22180. https://doi.org/10.34117/bjdv6n4-399.

GIAIA, 2017. Data from Fundão tailings dam failure. Available in: http://giaia.eco.br/materialdesuporte/.

Goldwasser, P., Feldman, J., 1997. Association of serum albumin and mortality risk. J. Clin. Epidemiol. 50, 693–703. https://doi.org/10.1016/s0895-4356(97)00015-2.

Guirlet, E., Dasb, K., Girondot, M., 2008. Maternal transfer of trace elements in leatherback turtles (Dermochelys coriacea) of French Guiana. Aquat. Toxicol. 88, 267–276. https://doi.org/10.1016/j.aquatox.2008.05.004.

Hamann, M., Jessop, T.S., Limpus, C.J., Whittier, J.M., 2005. Regional and annual variation in plasma steroids and metabolic indicators in female green turtles, Chelonia mydas. Mar. Biol. 148, 427–433. https://doi.org/10.1007/s00227-005-0082-6.

IBAMA, 2006. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Relatório Final da Proposta de Criação das UC's APA Costa das Algas e REVIS de Santa Cruz. Vitória, Volumes I, II, III, IV e V. Anexos 1 a 29.

IGAM, 2015. Relatório Técnico Acompanhamento da Qualidade das Águas do Rio Doce Após o Rompimento da Barragem da Samarco no distrito de Bento Rodrigues – Mariana/MG. Available in: http://www.igam.mg.gov.br/component/content/article/16/1632-monitoramento-da-qualidade-das-aguas-superficiais-do-rio-doce-no-estado-de-minas-gerais.

Irizarry-Rovira, A.R., 2004. In: Cowell, R.L. (Ed.), Veterinary clinical pathology secrets. Ed. Saunders Elsevier Inc. Mosby, Missouri, pp. 314–336.

Jacobson, E.R., 1993. Blood collection technics in reptiles: Laboratory investigations. In: Fowler, M.E. (Ed.), Zoo and Wild Animal Medicine: Current Therapy 3. W.B. Sawnder, Philadelphia, pp. 144–152.

Klaphake, E., 2010. A fresh look at metabolic bone diseases in reptiles and amphibians. Vet. Clin. North. Am. Exot. Anim. Pract. 13, 375–392. https://doi.org/10.1016/j.cvex.2010.05.007.

Kramer, J.W., Hoffmann, W., 1997. Clinical enzymology. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L. (Eds.), Clinical Biochemistry of Domestic Animals. Academic Press, San Diego, CA, pp. 303–325.

Kölle, P., Hoffmann, R., 2001. Renal diseases in reptiles: diagnostic tools. Proc. Abstr. EAZA Scientific Meeting, pp. 67–69.

Komoroske, L.M., Lewison, R.L., Seminoff, J.A., Deheyn, D.D., Dutton, P.H., 2011. Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. Chemosphere 84, 544–552. https://doi.org/10.1016/j.chemosphere.2011.04.023.

Komoroske, L.M., Lewison, R.L., Seminoff, J., Deustchman, D.D., Deheyn, D.D., 2012. Trace metals in an urbanized estuarine seaturtle food web in San Diego Bay, CA. Sci. Total Environ. 17, 108–116. https://doi.org/10.1016/j.scitotenv.2011.12.018.

Labrada-Martagón, V., Méndez-Rodríguez, L.C., Gardner, S.C., Cruz-Escalona, V.H., Zenteno-Savín, T., 2010. Health indices of the green turtle (Chelonia mydas) along the Pacific coast of Baja California Sur, Mexico. I. Blood biochemistry values. Chelonian Conserv. Biol. 9, 162–172. https://doi.org/10.2744/CCB-0806.1.

Labrada-Martagón, V., Rodríguez, P.A.T., Méndez-Rodríguez, L.C., Zenteno-Savín, T., 2011. Oxidative stress indicators and chemical contaminants in East Pacific green turtles (Chelonia mydas) inhabiting two foraging coastal lagoons in the Baja California peninsula. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 154, 65–75. https://doi.org/10.1016/j.cbpc.2011.02.006.

Ley-Quiñónez, C.P., Rossi-Lafferriere, N.A., Espinoza-Carreon, T.L., Hart, C.E., Peckham, S.H., Aguirre, A.A., Zavala-Norzagaray, A.A., 2017. Associations between trace elements and clinical health parameters in the North Pacific loggerhead sea turtle (Caretta caretta) from Baja California Sur, Mexico. Environ. Sci. Pollut. Res. 24, 9530–9537. https://doi.org/10.1007/s11356-017-8556-x.

Limpus, C.J., 1992. Estimation of tag loss in marine turtle research. Wildl. Res. 19, 457–638. https://doi.org/10.1071/WR9920457.

Limpus, C.J., Limpus, D.J., 2000. Mangroves in the diet of Chelonia mydas in Queensland, Australia. Mar. Turtle Newsl. 89, 13–15.

Limpus, C.J., 2008. A biological review of australian marine turtle species. 2. Green Turtle, Chelonia mydas (Linnaeus). Environmental Protection Agency, Brisbane.

Losey, G.S., Balazs, G.H., Privitera, L.A., 1994. Cleaning symbiosis between the wrasse, Thalassoma duperry, and the green turtle, Chelonia mydas. Copeia, 684–690 https://doi.org/10.2307/1447184.

López-Mendilaharsu, M., Gardner, S.C., Seminoff, J.A., Riosmena-Rodriguez, R., 2005. Identifying critical foraging habitats of the green turtle (Chelonia mydas) along the Pacific coast of the Baja California peninsula, Mexico. Aquat. Conserv. 15, 259–269. https://doi.org/10.1002/aqc.676.

Mader, D.R., 2005. Reptile Medicine and Surgery. Elsevier Health Sciences, St. Louis, USA 1241 p.

Maffucci, F., Caurant, F., Bustamante, P., Bentivegna, F., 2005. Trace element (Cd, Cu, Hg, Se, Zn) accumulation and tissue distribution in loggerhead turtles (Caretta caretta) from the Western Mediterranean Sea (southern Italy). Chemosphere 58 (5), 535–542. https://doi.org/10.1016/j.chemosphere.2004.09.032.

Mafra, D., 2003. Minerals and chronic renal disease. J. Bras. Nefrol. 25, 17–24.

Magris, R.A., Marta-Almeida, M., Monteiro, J.A.F., Ban, N.C., 2019. A modelling approach to assess the impact of land mining on marine biodiversity: assessment in coastal catchments experiencing catastrophic events (SW Brazil). Sci. Total Environ. 659, 828–840. https://doi.org/10.1016/j.scitotenv.2018.12.238.

Marcon, M., Gitirana, H.M., Werneck, M.R., Lobo-Hajdu, G., 2015. Hematological values of juvenile green turtles (Chelonia mydas) captured in the coast of Angra dos Reis and Paraty, South of Rio De Janeiro State. 46th Annual IAAAM Meeting and Conference, Chicago IL.

Marta-Almeida, M., Mendes, R., Amorim, F.N., Cirano, M., Dias, J.M., 2016. Fundão dam collapse: oceanic dispersion of river doce after the greatest brazilian environmental accident. Mar. Pollut. Bull. 112, 359–364. https://doi.org/10.1016/j.marpolbul.2016.07.039.

Masat, R.J., Dessauer, H.C., 1968. Plasma albumin in reptiles. Comp. Biochem. Phys. 25, 119–122. https://doi.org/10.1016/0010-406x(68)90918-3.
MB, 2016. Relatório de levantamento hidroceanográfico da Marinha do Brasil, Navio de pesquisa hidroceanográfico "Vital de Oliveita". Available in: http://agenciabrasil.ebc.com.br/sites/_agenciabrasil2013/files/files/Levantamento_Ambiental_Marinha.pdf.
McDermid, K.J., Stuercke, B., Balazs, G.H., 2007. Nutritional composition of marine plants in the diet of the green sea turtle (Chelonia mydas) in the Hawaiian Islands. Bull. Mar. Sci. 81, 55–71.
Mello, D.M.D., Alvarez, M.C.L., 2019. Health assessment of juvenile green turtles in southern São Paulo state, Brazil: a hematologic approach. J. Vet. Diagn. Investig. 32, 25–35. https://doi.org/10.1177/1040638719891972.
Miguel, C., Santos, M.R.D., 2019. Ecotoxicological studies of metal pollution in sea turtles of Latin America. In: Gómez-Oliván, L.M. (Ed.), Pollution of Water Bodies in Latin America. Springer, Cham, pp. 129–156 https://doi.org/10.1007/978-3-030-27296-8_9.
Mirlean, N., Garcia, F., Baisch, P., Quintana, G.C., Agnes, F., 2013. Sandy beaches contamination by arsenic, a result of nearshore sediment diagenesis and transport (Brazilian coastline). Estuar. Coast. Shelf Sci. 135, 241–247. https://doi.org/10.1016/j.ecss.2013.10.020.
Murray, S.N., Littler, M.M., 1978. Patterns of algal succession in a perturbated marine intertidal community. J. Phycol. 14, 506–512. https://doi.org/10.1111/j.1529-8817.1978.tb02477.x.
Musick, J., Limpus, C., 1997. Habitat utilization and migration in juvenile sea turtles. In: Lutz, P., Musick, J. (Eds.), The Biology of Sea Turtles. CRC Press, Boca Raton, FL, pp. 137–163.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858. https://doi.org/10.1038/35002501.
Norton, T.M., Wyneken, J., 2015. Body Condition Scoring the Sea Turtle. Lafeber Vet. Available in: http://lafeber.com/vet/body-condition-scoring-the-sea-turtle/.
Oliveira-Junior, A.A., Tavares-Dias, M., Marcon, J.L., 2009. Biochemical and hematologic reference ranges for Amazon freshwater turtle, Podocnemis expansa (Reptilia: Pelomedusidae), with morphologic assessment of blood cells. Res. Vet. Sci. 86, 146–151. https://doi.org/10.1016/j.rvsc.2008.05.015.
Osborne, A.G., Jacobson, E.R., Bresette, M.J., Singewald, D.A., Scarpino, R.A., Bolten, A.B., 2010. Reference intervals and relationships between health status, carapace length, body mass, and water temperature and concentrations of plasma total protein and protein electrophoretogram fractions in Atlantic loggerhead sea turtles and green turtles. J. Am. Vet. Med. Assoc. 237, 561–567. https://doi.org/10.2460/javma.237.5.561.
Owens, D.W., Ruiz, G.J., 1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36, 17–20.. https://www.jstor.org/stable/3891847.
Perrault, J.R., Stacy, N.I., Lehner, A.F., Poor, S.K., Buchweitz, J.P., Walsh, C.J., 2017. Toxic elements and associations with hematology, plasma biochemistry, and protein electrophoresis in nesting loggerhead sea turtles (Caretta caretta) from Casey Key, Florida. Environ. Pollut. 231, 1398–1411. https://doi.org/10.1016/j.envpol.2017.09.001.
Prioste, F.E.S., Souza, V.C.O., Queiroz, M.R., Chiacchio, R.G.D., Barbosa, F., Matushima, E.R., 2015. Chemical element concentrations in the blood of green turtles (Chelonia mydas) captured at Fernando De Noronha Marine National Park, Brazil. J. Environ. Anal Toxicol. 5, 1–5. https://doi.org/10.4172/2161-0525.1000325.
Prioste, F.E.S., 2016. Detection and quantification of selected inorganic chemical elements in blood and tissue samples from green turtles – Chelonia mydas (Linnaeus, 1758) – from the Brazilian coast: possible correlation with fibropapillomatosis. ThesisUniversidade de São Paulo 115 p.
Queiroz, H.M., Nóbrega, G.N., Ferreira, T.O., Almeida, L.S., Romero, T.B., Santaella, S.T., Bernardino, A.F., Otero, X.L., 2018. The samarco mine tailing disaster: a possible time-bomb for heavy metals contamination? Sci. Total Environ. 637–638, 498–506. https://doi.org/10.1016/j.scitotenv.2018.04.370.
Read, M.A., Limpus, C.J., 2002. The green turtle, Chelonia mydas, in Queensland: feeding ecology of immature turtles in Moreton Bay, southeastern Queensland. Mem. Queensl. Mus. 48, 207–214.
Register, A.L., 2011. Effects of heavy metal pollution on the loggerhead sea turtle. Loma Linda University, pp. 1–117 Master's Thesis.
Rossi, S., Zwarg, T., Sanches, T.C., Cesar, M.O., Werneck, M.R., Matushima, E.R., 2009. Hematological profile of Chelonia mydas (Testudines, Cheloniidae) according to the severity of fibropapillomatosis or its absence. Pesq. Vet. Bras. 29, 974–978. https://doi.org/10.1590/S0100-736X2009001200004.
Rossi, S., Sánchez-Sarmiento, A.M., Vanstreels, R.E.T., dos Santos, R.G., Prioste, F.E.S., Gattamorta, M.A., Grisi-Filho, J.H.H., Matushima, E.R., 2016. Challenges in evaluating the severity of fibropapillomatosis: a proposal for objective index and score system for green sea turtles (Chelonia mydas) in Brazil. PLoS ONE 11, e0167632. https://doi.org/10.1371/journal.pone.0167632.
Samarco, 2016. Atualização do Plano de Recuperação Ambiental Integrado. http://www.samarco.com/wp-content/uploads/2016/10/prai-agosto.pdf.
Santos, M.R.D., Ferreira, L., Batistote, C., Grossman, A., Bellini, C., 2009. Haematological values of wild juvenile turtles Chelonia mydas (Linaeus, 1758) from the Fernando de Noronha archipelago, Pernambuco, Brazil. Braz. J. Vet. Res. An. Sci. 46, 491–499. https://doi.org/10.11606/S1413-95962009000600008.
Santos, M.R.D., Martins, A.S., Baptistotte, C., Work, T., 2015. Health condition of juvenile Chelonia mydas related to fibropapillomatosis in Southeast Brazil. Dis. Aquat. Org. 115, 193–201. https://doi.org/10.3354/dao02883.
Santos, R.G., Martins, A.S., Nobrega Farias, J., Horta, P.A., Pinheiro, H.T., Torezani, E., Baptistotte, C., Seminoff, J.Á., Balazs, G.H., Work, T.M., 2011. Coastal habitat degradation and green sea turtle diets in southeastern Brazil. Mar. Pollut. Bull. 62, 1297–1302. https://doi.org/10.1016/j.marpolbul.2011.03.004.
Silva, A.E., Quaresma, V.S., Bastos, A.C., 2013a. Sedimentological sectorization of an estuarine system in a regressive coast, Southeast Brazil. J. Sediment. Res. 83, 994–1003. https://doi.org/10.2110/jsr.2013.78.
Silva, A.S., Leão, Z.M.A.N., Kikuchi, R.K.P., Costa, A.B., Souza, J.R.B., 2013b. Sedimentation in the coastal reefs of abrolhos over the last decades. Cont. Shelf Res. 70, 159–167. https://doi.org/10.1016/j.csr.2013.06.002.
Silva, C.C., Klein, R.D., Barcarolli, I.F., Bianchini, A., 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles Chelonia mydas from the southern Atlantic Ocean. Aquat. Toxicol. 170, 42–51. https://doi.org/10.1016/j.aquatox.2015.11.007.
Stamper, M.A., Harms, C., Epperly, S.P., Stoskopf, M.K., Braun-McNeill, J., 2005. Relationship between barnacle epibiotic load and hematologic parameters in loggerhead sea turtles (Caretta caretta), a comparison between migratory and residential animals in Pamlico Sound, North Carolina. J. Zoo Wildlife Med. 36, 635–641. https://doi.org/10.1638/04-074.1.
Stegeman, J.J., Brouwer, M., Di Giulio, R.T., Förlin, L., Fowler, B.A., Sanders, B.M., Van Veld, P.A., 1992. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In: Huggett, R.J., Kimerle, R.A., Mehrle Jr., P.M., Bergman, H.L. (Eds.), Biomarkers. Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, pp. 235–335.
Sykes, J.M., Klaphake, E., 2015. Reptile hematology. Clin. Lab. Med. 35, 661–680. https://doi.org/10.1016/j.cll.2015.05.014.
Swimmer, J.Y., 2000. Biochemical responses to fibropapilloma and captivity in the green turtle. J. Wildl. Dis. 36, 102–110. https://doi.org/10.7589/0090-3558-36.1.102.
Tauer, A.M., Liles, M.J., Chavarría, S., Valle, M., Amaya, S., Quijada, G., Meléndez, O., Rodríguez, S., Lock, E.F., Henríquez, A.V., Gaos, A.R., Seminoff, J.A., 2017. Hematology, biochemistry, and toxicology of wild hawksbill turtles (Eretmochelys imbricata) nesting in mangrove estuaries in the eastern Pacific Ocean. BioRxiv, 238956 https://doi.org/10.1101/238956.
Theml, H.K., Haferlach, T., Diem, H., 2004. Color atlas of hematology: Practical microscopic and clinical diagnosis. Thieme, Stuttgard/New York, p. 208.
Thrall, M.A., 2006. Veterinary hematology and clinical chemistry. Blackwell Publishing, Ames, Iowa, p. 776.
Thomson, J.A., Burkholder, D., Heithaus, M.R., Dill, L.M., 2009. Validation of a rapid visual-assessment technique for categorizing the body condition of green turtles (Chelonia mydas) in the field. Copeia 251–255. https://doi.org/10.1643/CE-07-227.
Van de Merwe, J.P., 2008. Persistent organic pollutants and heavy metals in the green sea turtle, Chelonia mydas. ThesisGriffith University, Queensland, Australia 284 p.
Van de Merwe, J.P., Hodge, M., Olszowy, H.A., Whittier, J.M., Lee, S.Y., 2010. Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (Chelonia mydas). Mar. Pollut. Bull. 60, 579–588. https://doi.org/10.1016/j.marpolbul.2009.11.006.
Vasconcelos, M.J.O.V., 2014. Os corais construtores da estrutura holocênica do recife da Coroa Vermelha, Abrolhos, Bahia. DissertationUniversidade Federal da Bahia, Brasil 109 p.
Villa, C.A., Flint, M., Bell, I., Hof, C., Limpus, C.J., Gaus, C., 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. Environ. Pollut. 220, 1465–1476. https://doi.org/10.1016/j.envpol.2016.10.085.
Villegas-Nava, F.E., 2006. Análisis nutricional de macroalgas y pastos asociados a la alimentación de tortuga prieta Chelonia mydas agassizii (Bocourt 1968), en Bahía Magdalena, B.C.S., México. ThesisUniversidad Autónoma de Baja California Sur, Mexico 65 p.
Walsh, M., 1999. Rehabilitation of sea turtles. In: Eckert, K.L., Abreu-Grobois, F.A., Donnelly, M. (Eds.), Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4, Washington, DC, pp. 200–207.
Whiting, S.D., Guinea, M.L., Limpus, C.J., Fomiatti, K., 2007. Blood chemistry reference values for two ecologically distinct populations of foraging green turtles, eastern Indian Ocean. Comp. Clin. Path. 16, 109–118. https://doi.org/10.1007/s00580-006-0646-y.
Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., Watson, R., 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314, 787–790. https://doi.org/10.1126/science.1132294.
Work, T.M., Balazs, G.H., 1999. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. J. Wildl. Dis. 35, 804–807. https://doi.org/10.7589/0090-3558-35.4.804.
Work, T.M., Rameyer, R.A., Balazs, G.H., Cray, C., Chang, S.P., 2001. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. J. Wildl. Dis. 37, 574–581. https://doi.org/10.7589/0090-3558-37.3.574.
Zhang, F., Gu, H., Li, P., 2011. A review of chelonian hematology. Asian Herp. Res. 2, 12–20. https://doi.org/10.3724/SP.J.1245.2011.00012.
Zwarg, T., Rossi, S., Sanches, T.C., Cesar, M.O., Werneck, M.R., Matushima, E.R., 2014. Hematological and histopathological evaluation of wildlife green turtles (Chelonia mydas) with and without fibropapilloma from the north coast of São Paulo state, Brazil. Pesq. Vet. Bras. 34, 682–688 c.