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Metal accumulation in female green sea turtles (*Chelonia mydas*) from Eastern Atlantic affects their egg quality with potential implications for embryonic development

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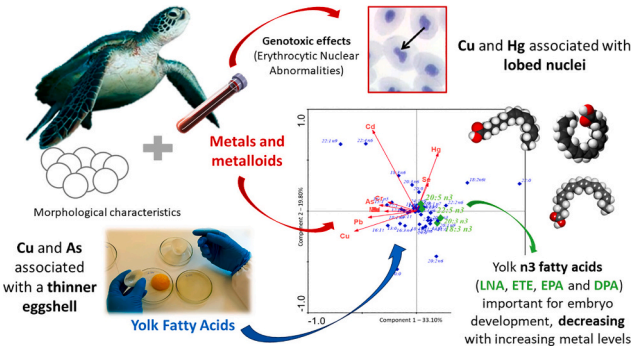
HIGHLIGHTS

- Cu and Hg levels associated with erythrocytes' abnormalities in nesting females.
- Increased Cu and As levels associated with a decrease in eggshell thickness.
- Pollution load index links consistently with a decrease of vital FAs in egg yolk.
- Increased metal levels associated with a decrease in the quality of egg reserves.
- Metals in female sea turtles may impair embryonic development of their offspring.

GRAPHICAL ABSTRACT



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ABSTRACT

Sea turtles, with their global distribution and complex life cycle, often accumulate pollutants such as metals and metalloids due to their extended lifespan and feeding habits. However, there are limited studies exploring the impact of metal pollution on the reproductive health of female sea turtles, specifically focusing on the quality of their eggs, which has significant implications for the future generations of these charismatic animals. São Tomé Island, a crucial nesting and feeding habitat for green sea turtles, underscores the urgent need for comprehensive research in this ecologically significant area. This study aimed to investigate whether metals and metalloids in the blood of nesting female green sea turtles induce genotoxic effects in their erythrocytes and affect their egg morphometric characteristics and the composition of related compartments. Additionally, this study aimed to evaluate whether the quality of energetic reserves for embryo development (fatty acids in yolk's polar and

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neutral lipids) is influenced by the contamination status of their predecessors. Results revealed correlations between Cu and Hg levels and increased "lobed" erythrocytes, while As and Cu negatively influenced shell thickness. In terms of energy reserves, both polar and neutral lipid fractions contained primarily saturated and monounsaturated fatty acids, with prevalent 18:1n-9, 18:0, 16:0, 14:0, and 12:0 fatty acids in yolk samples. The yolk polar fraction was more susceptible to contaminant levels in female sea turtles, showing consistent negative correlations between pollution load index and essential n3 fatty acids, including linolenic, eicosatrienoic, eicosapentaenoic, and docosapentaenoic acids, crucial for embryonic development. These metals accumulation, coupled with the reduced availability of these key fatty acids, may disrupt the eicosanoid and other important pathways, affecting reproductive development. This study reveals a negative correlation between metal contamination in female sea turtles' blood and egg lipid reserves, raising concerns about embryonic development and the species' future generations.

1. Introduction

In recent years, there has been extensive documentation of the significant impact of anthropogenic pressures on global biodiversity (Mach et al., 2015). This impact is primarily attributed to the diverse range of contaminants generated by human activities, which have indiscriminately affected terrestrial and marine ecosystems (Tanabe et al., 2022). While metals and metalloids are naturally present in marine ecosystems, human activities have led to an escalation in their concentrations within the environment (Silva et al., 2017; Morão et al., 2022). Metals and metalloids encompass both essential elements, such as iron (Fe), selenium (Se), and zinc (Zn), as well as non-essential elements, including chromium (Cr), cadmium (Cd), lead (Pb), and mercury (Hg). While essential metals are required by organisms in small amounts, non-essential metals can be toxic even at low concentrations (Filimonova et al., 2016). These contaminants persist in the environment and within biological systems, undergoing processes of bioaccumulation and biomagnification. As a result, they have the potential to amass in elevated concentrations at the upper levels of the food chain, endangering apex predators like marine mammals (López-Berenguer et al., 2020) and sharks (Alves et al., 2023). Additionally, various other organisms, including sea turtles (Ross et al., 2017), face potential threats due to these accumulated concentrations. The deleterious effects of metals and metalloids have been extensively demonstrated across various ecosystems, exhibiting impacts at different levels of biological organisation. These effects include hatching delays, deformities, and mortality in fish larvae (Sfakianakis et al., 2015), as well as oxidative stress and apoptosis in fish, resulting in tissue damage (Alves et al., 2016; Morcillo et al., 2016). Additionally, studies on sea turtle cell lines have revealed cytotoxic, oxidative, and genotoxic effects, affecting cellular health (Finlayson et al., 2019; Johnson et al., 2022; Speer et al., 2018) and gene expression (Cortés-Gómez et al., 2018; Morão et al., 2022). One effective approach to evaluate genotoxic effects caused by contamination is through the assessment of different blood parameters, including erythrocytic nuclear abnormalities (ENAs). This parameter provides valuable insights into the physiological condition of organisms (Casini et al., 2018; Morão et al., 2022), enabling the detection of genotoxic effects, such as chromatin fragmentation leading to micronucleus formation (Zapata et al., 2016).

Sea turtles are globally migratory animals with a complex life cycle that encompasses multiple environments (Carr, 1982). Six out of the seven existing sea turtle species are included in the IUCN Red List as threatened species (Godfrey and Godley, 2008). Owing to their long lifespan and feeding habits, these reptiles have a propensity for accumulating metals and metalloids in their tissues (Yipel et al., 2017; Morão et al., 2022). However, limited knowledge exists regarding the potential impacts of metal pollution on the overall health of reproductive female sea turtles and especially on the quality of their eggs (Barraza et al., 2021), which could have implications for the well-being of sea turtles (Guirlet et al., 2008; Páez-Osuna et al., 2010).

Lipids are highly dynamic molecules that exhibit great susceptibility to environmental changes. Metals and metalloids can influence the composition and content of lipids, thus making these modifications suitable for monitoring the toxicological aspects of ecosystem health (Gonçalves et al., 2017; Silva et al., 2021). Fatty acids (FA) are important cell membrane components and signalling mediators involved in a myriad of biological pathways, having vital rules in detoxification and inflammatory responses (Silva et al., 2021). Fatty acids also have a critical role in embryo development providing the necessary energy and nutrition for proper embryo growth and development (Dunning et al., 2014; McKeegan and Sturmey, 2011). Only a few studies have addressed the effects of metals and metalloids on FA profiles and lipid pathways in marine vertebrates. Although there are no studies showing these effects in sea turtles, this interaction has been mostly studied in fish (Kaur and Brraich, 2022). Additional effects have been documented in diatoms (Duarte et al., 2022), as well as in invertebrate species including sea snails (Silva et al., 2017, 2021), shrimps (Silva et al., 2021) or bivalve species (Fokina et al., 2013), where metal levels induced alterations in their fatty acid profiles, potentially influencing inflammatory and immune regulation responses.

In reptiles, including sea turtles, the egg yolk constitutes a significant portion of the egg mass and is characterised by its high lipid content, accounting for approximately 13 % of the yolk composition (Hewavisenthi and Parmenter, 2002). Additionally, yolk fatty acids are the major source of energy for embryo development and for the post-hatching activity, as both metabolic and signalling mediators (Guirlet et al., 2008; Lawniczak and Teece, 2009). Furthermore, metals and metalloids have been found in both eggs and hatchlings of sea turtles, which possibly poses deleterious effects on their well-being (Páez-Osuna et al., 2010; Perrault et al., 2011; Souza et al., 2018). Due to their life cycle and egg/embryo development, sea turtle embryos and hatchlings are susceptible to metal accumulation (Tanabe et al., 2022; van de Merwe et al., 2009). They tend to accumulate these contaminants both through vertical transference and environmental exposure (e.g. Guirlet et al., 2008; Páez-Osuna et al., 2011), posing a threat to the viability of future generations of these endangered species.

São Tomé Island holds important nesting and feeding grounds for four of the seven existing species of sea turtles, including the green sea turtle (*Chelonia mydas*) (Ferreira-Airaud et al., 2022; Morão et al., 2022). The nesting population of green sea turtles of São Tomé and Principe archipelago is recognised as a genetically distinct rookery, exhibiting relatively high levels of genetic diversity and distinctiveness, representing an important genetic pool in the region (Formia et al., 2006; Hancock et al., 2019). Therefore, understanding the pollution impacts on this population is crucial. This knowledge is not only essential for gaining insights into the potential effects of metal contamination across generations but also to providing valuable information for policy and conservation initiatives in the country, such as "Programa Tatô" in São Tomé Island and "Fundação Principe" in Príncipe Island, aimed at preserving sea turtles and their habitats.

Thus, this study had the following main goals: 1) to evaluate if metal levels in the blood of nesting green sea turtles can be inducing genotoxic effects in those female turtles; 2) to explore if these contamination levels could be associated with alterations in their egg morphometric characteristics and composition, including the respective compartments (e.g., total fat, weight from the different compartments, shell thickness and

egg dimensions); 3) to assess if the quality of the energetic reserves for embryo development (fatty acids in the yolk) can be affected by the contamination status of the nesting females.

2. Material and methods

This work was ethically approved by the "Direcção Geral do Ambiente (DGA)" of São Tomé and Príncipe (STP) and by the "Instituto da Conservação da Natureza e das Florestas (ICNF)" of Portugal. The samples were imported to Portugal (Polytechnic of Leiria) under CITES permission 18ST000001/AC,18PTLX00159I.

2.1. Study site and sampling

Female green turtles were sampled at night time during nesting activity at Jalé beach (0°03′16.6″N, 6°30′54.5″E) in the south of São Tomé Island (859 km^2) (0°20′0″N,6°44′0″E), in the nesting season of 2017/2018 as previously detailed in Morão et al. (2022).

Briefly, twenty-seven fresh laid eggs (one per female) were sampled when the females had already layed about 20 eggs, to decrease the possibility of the females abandoning the nest, and trying to prevent them to contact the sand. The eggs were immediately placed in a plastic zip lock bag (individualised) and kept at −20 °C until morphometry measurements (Section 2.2) and yolk lipid reserves analysis (Section 2.3) were performed.

Additionally, the sampling included the collection of twenty-seven blood samples, from the same female individuals, as previous described in Morão et al. (2022). The collected blood was also used for metal and metalloid analysis (Section 2.4). Simultaneously, during the blood collection, two blood smears per female were prepared on slides using one drop of blood. After drying, the smears were fixed with hair lacquer and stored for genotoxic analysis (Section 2.5).

2.2. Egg morphometry

Eggs were rinsed with deionised water (Milli-Q Millipore, Merck) to remove any potentially attached particulate matter or sand (Páez-Osuna et al., 2010). Then, all eggs were weighed (±0.01 g) and measured with a Vernier calliper (Avantor, 819–0012) with a precision of 0.05 mm (±0.01). Both measuring and weighing processes were repeated three times for accuracy. Subsequently, each egg was manually separated into shell, membrane, albumen, and yolk, with individual weights recorded (±0.01 g).

Prior to visual inspection and shell thickness measurement, each shell underwent preparation. Two clean cuts were done in different shell sections to examine non-uniform areas, ensuring more accurate results. The handling and processing of turtle eggs were carried out using a ZEISS STEMI 2000-C trinocular stereo zoom microscope (4× or 3.2× magnification) (Carl Zeiss Microimaging GmbH Göttingen, Germany). Afterwards, the AxioCam MRc camera (Carl Zeiss Microscopy GmbH, Göttingen, Germany) was used to capture the images and the Zen 2011 application (blue edition) software was used to perform the measurements (scale 200 μm).

For the lipid analysis, egg yolks were mechanically homogenised and separated into aliquots of 2 g for posterior lipid extraction and quantification (Section 2.3.1) and fatty acid profiling (Sections 2.3.2 and 2.3.3).

2.3. Egg lipid composition

2.3.1. Total lipid extraction and quantification

Total lipids from the egg yolks were extracted and quantified using the established methodology of Bligh and Dyer (1959) with minor modifications. A volume of 9 mL of the mixture methanol/chloroform (2:1 v/v) was added to 2 g of yolk, previously separated, and the homogenate was stirred for 3–5 min at room temperature. After, 3 mL of saturated sodium chloride (NaCl) solution (40 %) was added to each sample and stirred again (1 min). Afterwards, 3 mL of Milli-Q water and 3 mL of chloroform were added, and samples were stirred again for 2 min before centrifugation at 4500 ×*g* for 10 min. The organic bottom layer was collected and filtered through an anhydrous sodium sulphate (Na_2SO_4) column to pear-shaped flasks. The solvent was then evaporated at 40 °C in a rotary evaporator (Heidolph, Laborota-4000, Germany). Samples were weighed, and the percentage of the total fat in each sample was calculated according to the Eq. (1):

$$\text{Total Fat}\ (\%) = \frac{\text{f w} - \text{i w}}{\text{s W}} \times 100 \quad (1)$$

where iW is the initial weight of the empty flasks (g), fW is the final weight of the flasks with the lipidic fraction (g), and sW is the initial sample weight (g).

2.3.2. Lipid fractionation

Before the methylation process for fatty acid profile analysis (Section 2.3.3), an additional separation of polar and neutral lipids was made by Thin Layer Chromatography (TLC) (Donato et al., 2017). Here, 150 mg of total fat was dissolved in 6.25 mL of chloroform, and 200 μL of this solution was spotted into preparative and activated silica TLC plates (TLC Silica Gel 60G 25 Glass plates 20, MERK). Lipid reference standards (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine, cholesterol, cholesterol oleate, tripalmitin) were used to initially identify the different categories of lipid compounds. The retention factor (R_f) values employed to measure the eluted bands along the TLC plate were similar between standards and sample compounds. This similarity allowed for the identification of groups of lipid compounds (Fig. S1). Lipids were eluted and separated with a mixture of n-hexane/diethyl ether/acetic acid (35.5/15/0.75, v/v/v) (Fuchs et al., 2011) as mobile phase to resolve the different lipids: Triacylglycerols (TAG), Phospholipids (PL), Cholesteryl esters (CE), and Cholesterol (C). Lipid spots were detected by spraying plates with a 0.2 % (w/v) solution of 2′, 7′-dichlorofluorescein in 95 % ethanol. The plates were then air-dried in a fume hood and observed under UV light at 366 nm. After the detection and identification of the different lipid categories, polar (PL) and neutral (TAG and CE) bands were scrapped and transferred to new tubes for methylation, to be assayed as fatty acid methyl esters (FAME) by gas chromatography (GC) (see Section 2.3.3).

2.3.3. Fatty acid profile

For the analysis of more complex lipids (phospholipids, PL), an initial saponification step was performed by adding 500 μL of 2 M KOH (diluted in 67 % ethanol; v/v) to the polar fraction obtained from the TLC plate of each sample. The samples were subsequently incubated at 80 °C for 1 h, followed by cooling to room temperature. They were then diluted 1:1 with water and acidified to pH = 1 using HCl (5 M). Next, FAs were isolated by adding 500 μL of hexane to the samples, which were then stirred and centrifuged at 1500×*g* for 5 min to collect the upper organic phase.

For the methylation step, 3 mL of acetyl chloride: methanol (1:20 v/v) solution was added both to the polar and neutral fractions. The samples were incubated at 80 °C for 1 h, after which 1 mL of Milli-Q water and 1 mL of n-heptane were added. Samples were then stirred during 1 min for phase separation to recover the organic layer and transfer it to clean GC vials. In the case of the polar fractions, the solvent was evaporated under a nitrogen stream to 100 μL of volume and transferred to clean GC vials with micro-inserts.

Gas chromatography operating conditions were as described by Silva et al. (2017, 2021) with brief modifications. A Finnigan Ultra Trace gas chromatograph equipped with a Thermo TR-FAME capillary column (60 m × 0.25 mm ID, 0.25 μm film thickness), an auto sampler AS 3000 from Thermo Electron Corporation and a flame ionization detector (FID) were used to analyse the fatty acids methyl esters. The injector

(operating in splitless mode) and the detector temperatures were set at 250 °C and 280 °C, respectively. The column temperature was initially set at 100 °C for 1 min, then raised 10 °C.min^{-1} until 160 °C and held for 10 min, followed by an increase of 4 °C.min^{-1} until 235 °C and maintained for 10 min. Helium was used as carrier gas at a flow rate of 1.5 mL.min^{-1}. Air and hydrogen were supplied to the detector at flowrates of 350 and 35 mL.min^{-1}, respectively. Fatty acid methyl ester mixes (PUFA No1 from marine source, PUFA No 3 from menhaden oil and a 37-component FAME mix) were used as external standards (Supelco, Bellefonte, PA, USA). Theoretical correction factor (FCT) for FID detectors was applied in FA quantification (Guo, 2014). FAs with four or more unsaturations in their aliphatic chain were considered as highly unsaturated FAs (HUFA). Results were expressed as mg.g^{-1} of total fatty acids.

2.4. Element quantification and pollution load index (PLI)

Several metal and metalloid elements [mercury (Hg), arsenic (As), lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), selenium (Se), iron (Fe), manganese (Mn), silver (Ag), and aluminium (Al)] were previously quantified in blood samples from the respective adult females as reported in Morão et al. (2022). The lyophilised blood used underwent an acidic digestion and were then screened for the specified elements previous mentioned using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Metal concentrations were expressed in μg.g^{-1} of blood dry weight (dw).

Pollution Load Index (PLI) was used as an index for the total assessment of the degree of contamination in sea turtles. It was adapted from the model initially proposed by Tomlinson et al. (1980), using Concentration Factors (CF) of each element (C metal) in sea turtle's blood. Reference intervals for many trace elements were established in the study conducted by Villa et al. (2017), and these were used as the baseline concentrations (C baseline) in this research. However, due to the lack of reference values for Al and Hg, the minimal value observed in the present study for these two elements was used as C baseline, as previously performed with aquatic organisms (Angulo, 1996; Tahity et al., 2022). The equations applied were as follows:

$$CF = \frac{\text{C metal}}{\text{C baseline}} \quad (2)$$

$$PLI = \sqrt[n]{CF1 \times CF2 \times CF3 \times \ldots \times CFn} \quad (3)$$

2.5. Erythrocytic nuclear abnormalities (ENAs) assay

Two blood smears per sample were prepared. The slides were stained with Diff-Quick stain. After fixed and stained, 1000 mature erythrocytes for each animal were analysed to score the different erythrocytic nuclear abnormalities (Casini et al., 2018; Morão et al., 2022). The nuclear lesions were scored into one of the following categories: micronuclei, lobed nuclei, segmented nuclei and kidney-shaped nuclei. The results were expressed as the mean value (‰) of each abnormality and the sum of all the observed lesions.

2.6. Statistical analysis

Data were summarised for each parameter using means and standard deviation (SD) values. The Shapiro-Wilk test was used to test data for normality. To compare classes, groups, and FAs ratios between the two fractions, a Paired Samples *t*-test was employed for samples exhibiting a normal distribution, while a Wilcoxon test was utilized for non-normally distributed data.

Correlations between egg morphometric characteristics, FA profile or genotoxicity biomarkers and element concentrations were analysed using Spearman's linear correlation coefficients. To describe the strength of each correlation, the guidelines proposed by Evans (1996) were employed. According to these guidelines, the absolute value of the correlation coefficient (r_s) falls into different categories: 0.00–0.19 is considered "very weak", 0.20–0.39 is considered "weak", 0.40–0.59 is "moderate", 0.60–0.79 is "strong", and 0.80–1.0 is considered "very strong". Canonical Correspondence Analysis (CCA) was performed to analyse the pattern of distribution and relation between metal concentrations and fatty acids profile. For this analysis, the data on metals concentration were standardised and transformed log (x + 1) (Hsu and Culhane, 2020). Downweighing of FA profile was performed to take into consideration the less representative FA (Lepš and Šmilauer, 2003). For all statistical analysis, the significance level was set at $P \leq 0.05$. Where applicable, results are expressed as mean ± standard deviation (SD). Correlation analysis and Paired Samplest test were performed using IBM SPSS Statistics 28.0.1.0 (IBM Corp, 2023). CCA was performed with CANOCO version 4.5 package 5 (ter Braak and Šmilauer, 2002).

3. Results

3.1. Egg morphometry and lipid content

The mean egg diameter measured was 39.80 ± 1.49 mm, with a corresponding whole egg weight of 33.90 ± 3.70 g. Additionally, the average shell thickness was 156.95 ± 25.95 μm. The yolk represented almost half of the egg weight and was on average 15.97 ± 2.10 g (Table 1), having in its composition 13.82 ± 2.83 % of Total Fat (TF) on average.

Regarding lipid content, egg yolks presented higher percentages of SAT fatty acids, followed by MUFA, HUFA and PUFA in both fractions (Table 2). For NF, similar percentages of SAT (47.73 %) and MUFA (46.24 %) were observed, while in the PF, the same classes presented 54.70 % and 25.33 %, respectively. The percentage of SAT and HUFA fatty acids was significantly higher in the PF fraction compared to the NF fraction (Table 2, $P < 0.001$). Conversely, for MUFA, higher values were observed in NF compared to PF ($P < 0.001$). PUFAs class presented similar values between the two lipid fractions.

Regarding the FA groups, the PF presented a higher unsaturation degree comparatively to the NF. The percentage of omega-3 (n3) and omega-6 (n6) fatty acids was significantly higher in the PF fraction than in the NF fraction (Table 2, $P < 0.001$). On the other hand, for the omega-7 (n7) and omega-9 (n9) groups, the opposite was observed, with higher and significant ($P < 0.001$) values in the NF fraction compared to the PF.

By analysing important ratios between the two fractions, higher values were observed for the NF in the n3/n6, SAT/HUFA, and MUFA/HUFA ratios (Table 2, $P < 0.001$). Conversely, the SAT/UNSAT ratio was higher in the PF fraction (Table 2, $P < 0.001$). The DHA/EPA ratio presented similar values between the two fractions ($P = 0.178$), indicating that these FAs are present in both fractions with the same proportions.

Globally, the yolk samples presented a fatty acid profile ranging from 6:0 (caproic acid) to 24:1 n9 (nervonic acid), comprising a total of 48 distinct FAs detected and identified throughout this study (Table S1 – supplementary material).

Table 1
Mean ± standard deviation and range values (minimum-maximum) of the egg's morphometric characteristics in female green sea turtles (*Chelonia mydas*).

Morphometric characteristics	Average ± SD (min – max)
Diameter (mm ± 0.01)	39.80 ± 1.49 (37.30–43.39)
Whole egg (g)	33.90 ± 3.70 (26.79–40.15)
Shell thickness (μm)	156.95 ± 25.95 (101.14–215.76)
Yolk (g)	15.97 ± 2.10 (11.90–20.34)
Shell (g)	2.66 ± 0.45 (1.97–3.43)
Albumin (g)	10.12 ± 3.17 (3.34–16.04)
Membrane (g)	3.60 ± 2.30 (0.57–12.96)
Yolk Total Fat (%)	13.82 ± 2.83 (8.20–18.27)

Table 2
Percentage composition for the different categories of fatty acids (mean ± SD) in the two fractions of egg yolk samples of green sea turtles (*Chelonia mydas*). Different important ratios for the fatty acid content analysis are also presented. SAT = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; HUFA = highly unsaturated fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; NF = neutral fraction with triacylglycerols and cholesteryl esters and PF = polar fraction with phospholipids. The statistic tests used were (a) – Shapiro-Wilk test and (b) – Wilcoxon test.

FA categories	Neutral fraction (NF)	Polar fraction (PF)	Significance values between fractions (P)
SAT	47.73 ± 0.68	54.70 ± 2.83	<0.001 (a)
MUFA	46.24 ± 0.66	25.33 ± 1.31	<0.001 (a)
PUFA	2.22 ± 0.03	2.02 ± 0.10	0.16 (b)
HUFA	3.80 ± 0.05	17.95 ± 0.93	<0.001 (b)
n3	2.74 ± 0.04	7.80 ± 0.40	<0.001 (a)
n6	3.15 ± 0.04	12.02 ± 0.62	<0.001(a)
n7	10.33 ± 0.15	4.21 ± 0.22	<0.001 (b)
n9	35.22 ± 0.50	19.70 ± 1.02	<0.001 (b)
trans	0.58 ± 0.01	1.30 ± 0.07	0.002 (b)
n3/n6	1.06 ± 0.61	0.69 ± 0.27	<0.001 (b)
DHA/EPA	1.21 ± 0.52	1.19 ± 0.71	0.18 (a)
SAT/UNSAT	0.92 ± 0.08	1.24 ± 0.24	<0.001 (b)
SAT/PUFA	24.62 ± 9.61	35.59 ± 23.62	0.046 (b)
SAT/HUFA	13.80 ± 4.69	3.24 ± 0.96	<0.001(b)
MUFA/PUFA	23.81 ± 8.81	16.58 ± 11.71	0.004 (b)
MUFA/HUFA	13.33 ± 4.43	1.47 ± 0.32	<0.001(b)

The analysis of the individual FAs demonstrated that yolk samples from green sea turtles contained mostly 18:1n9 (oleic acid, 333.29 ± 21.42 mg.g^{-1}), 12:0 (lauric acid, 166.49 ± 19.17 mg.g^{-1}), 16:0 (palmitic acid, 156.60 ± 11.73 mg.g^{-1}), 14:0 (myristic acid, 79.12 ± 7.70 mg.g^{-1}), 16:1n7 (palmitoleic acid, 58.97 ± 6.37 mg.g^{-1}), 18:0 (stearic acid, 45.57 ± 10.83 mg.g^{-1}) and 18:1n7 (vaccenic acid, 40.96 ± 9.02 mg.g^{-1}), which are the fatty acids that are present in higher proportions in the most representative fraction, NF (Table S1). PF was predominantly represented by 16:0 (palmitic acid, 218.86 ± 21.66 mg.g^{-1}), followed by 18:0 (stearic acid, 201.89 ± 24.04 mg.g^{-1}), 18:1n9 (oleic acid, 175.29 ± 55.24 mg.g^{-1}), 20:4 n6 (arachidonic acid, ARA, 97.44 ± 26.89 mg.g^{-1}), 12:0 (lauric acid, 35.42 ± 23.71 mg.g^{-1}), 18:1 n7 (vaccenic acid, 29.81 ± 43.59 mg.g^{-1}) and by 14:0 (myristic acid, 26.24 ± 8.15 mg.g^{-1}) (Table S1). The most common FAs present in the two fractions were 18:1n9, 16:0, 18:0 and 12:0 (Table S1). However, some other important FAs, such as 20:5 n3 (eicosapentaenoic acid, EPA, 21.95 ± 13.39 mg.g^{-1}) and 22:6 n3 (docosahexaenoic acid, DHA, 19.40 ± 5.14 mg.g^{-1}), although presenting similar values for their ratios, in PF were found in relevant proportions considering the range values observed for all fatty acids (0.05 to 218.86 mg.g^{-1}) (Table S1).

3.2. Element quantification and pollution load index (PLI)

Metal and metalloid levels found in the blood of the studied nesting females, retrieved from the previous publication of Morão et al. (2022), can be found in Table 3, along with the calculated concentration factors (CF) for each element needed to assess the overall degree of metal pollution in the present sea turtles by the pollution load index (PLI).

The analysis of CF values for each metal indicates that Al (68.07 ± 320.51) exhibited the highest values, followed by Mn (13.58 ± 48.75), Hg (10.45 ± 18.72), and Pb (8.46 ± 11.09). On the other hand, Cd (1.47 ± 2.45) showed the lowest CF value. The overall PLI for the sampled green sea turtles was 3.72 ± 6.54, with minimum value of 1.63 and maximum of 36.96 (more details can be found in Table S2).

Table 3
Mean ± standard deviation values of metal concentrations (expressed as μg.g^{-1} of dry weight), their corresponding concentration factor (CF) values and the overall pollution load index (PLI) determined in blood of green sea turtles (*Chelonia mydas*). <LOQ = Below limit of quantification; *≤0.001 mg.L^{-1}; NA = not applicable.

Metal	Metal concentration	Metal CF	PLI
Aluminium (Al)	5.03 ± 20.78	68.07 ± 320.51	
Arsenic (As)	2.07 ± 1.59	2.88 ± 3.30	
Lead (Pb)	0.97 ± 0.59	8.46 ± 11.09	
Cadmium (Cd)	0.03 ± 0.06	1.47 ± 2.45	
Chromium (Cr)	0.02 ± 0.04	5.43 ± 2.46	
Iron (Fe)	2189.59 ± 869.04	1.89 ± 2.31	
Copper (Cu)	3.79 ± 1.22	1.83 ± 1.63	3.72 ± 6.54
Manganese (Mn)	0.78 ± 1.76	13.58 ± 48.75	
Nickel (Ni)	<LOQ*	NA	
Mercury (Hg)	0.006 ± 0.012	10.45 ± 18.72	
Selenium (Se)	3.94 ± 4.48	3.94 ± 4.28	
Silver (Ag)	<LOQ*	NA	
Zinc (Zn)	91.30 ± 57.18	2.44 ± 4.01	

3.3. Effects of metal contamination on female green sea turtles' blood and eggs

3.3.1. Erythrocytic nuclear abnormalities

The frequencies of nuclear abnormalities in green sea turtles' erythrocytes are summarised in Table S3, along with the micronuclei (MN) for these same organisms, previously reported and described in Morão et al. (2022). Photomicrographic representation of erythrocytic nuclear abnormalities are showed in Fig. S2.

Among the nuclear abnormalities evaluated, those classified as lobed had the highest average frequency of 19.08 ± 14.90 ‰. The kidney-shaped nuclei followed with an average of 0.50 ± 0.69 ‰, and the segmented category had the lowest average occurrence of 0.08 ± 0.27 ‰ (Table S3). The total amount of abnormalities observed were in average 28.31 ± 16.07 ‰ (Table S3). It was found that higher levels of Cu (r_s = −0.49; P = 0.01) were associated with a decrease in the frequency of normal erythrocytes along with an increase in the occurrence of lobed erythrocytes (r_s = 0.44; P = 0.02; Table S4). Similarly, higher concentrations of Hg (r_s = 0.47; P = 0.01; Table S4) were associated with an increase in the occurrence of lobed nuclei in erythrocytes. In fact, a higher overall pollution load index (PLI) was correlated to a higher occurrence of lobed nuclei (r_s = 0.43; P = 0.03; Table S4).

3.3.2. Egg morphometry and lipid content

No significant correlations were observed between the analysed egg morphometric parameters and the Pollution Load Index (PLI). Contrarily, when analysing the effects of metals individually, it was found that shell thickness (μm) showed a moderate and negative correlation with As (r_s = −0.45; P = 0.02) and Cu (r_s = −0.43; P = 0.03) (Table S5).

3.3.3. Yolk fatty acid profile

In a first exploratory analysis, and to evaluate the relation between environmental contaminants present in females' blood and FAs profile in yolk samples, a Canonical Correspondence Analysis (CCA) was performed in both lipidic fractions (Fig. 1), considering the relations of contaminant levels with classes of FAs (Fig. 1A, C) and individual FAs (Fig. 1B, D).

When analysing the influence of the contaminants in FA classes (Fig. 1A, C) a similar pattern is observed between the two lipidic fractions, with PUFA being more negatively influenced by the presence of contaminants. MUFA seem to be less influenced by metal and metalloid levels, since non-significant correlations were found for this FAs class with element levels in both fractions (Tables S6 and S7). However, when looking at the influence of contaminants to individual fatty acids (Fig. 1B, D), it can be observed that in the NF (Fig. 1B) FAs are less

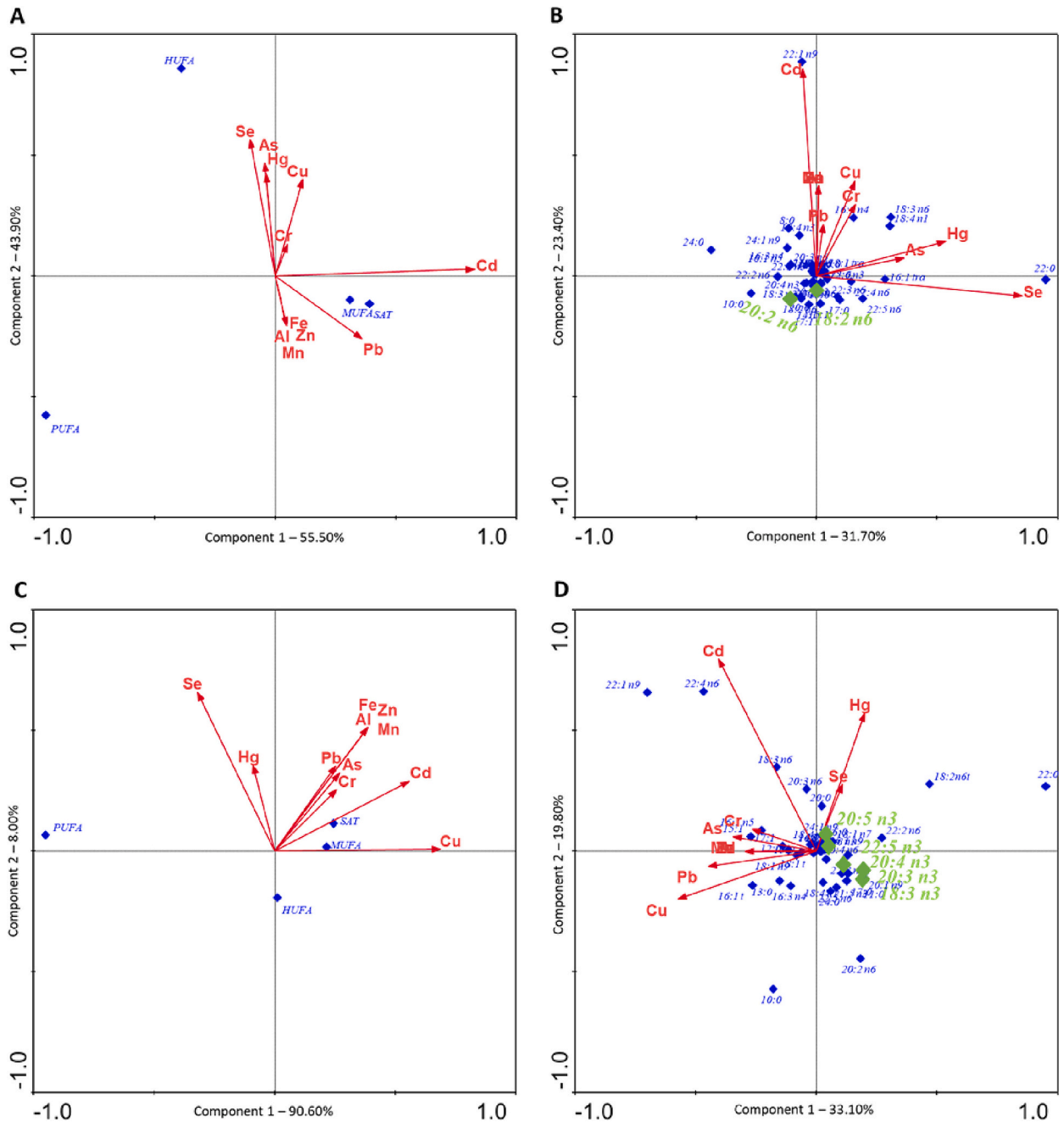

Fig. 1. Biplots of axes 1 and 2 of the Canonical Correspondence Analysis (CCA) between metal and metalloid levels in the blood of female green sea turtles (*Chelonia mydas*) and fatty acid profile in their egg yolk samples. (A) and (C) represent neutral and polar fractions of fatty acid classes. (B) and (D) represent individual fatty acids in those fractions, respectively. Red arrows represent metals, blue diamonds represent classes or individual FAs. The most representative fatty acids are highlighted in green.

dispersed and concentrated in the centre of the biplot, indicating that the metals are not having a great influence on the variability of the FA data, which can also be seen by the correlation analyses (Table S6) where less specific and more spread out correlations between groups of FAs were observed. The exception was with specific n6 fatty acids 18:2 n6 (linoleic acid, LA) and 20:2 n6 (eicosadienoic acid, EDA) that showed strong and negative correlations with different contaminants (LA vs Cu: $r_s = -0.59$, $P < 0.001$; LA vs Hg: $r_s = -0.48$, $P = 0.01$; LA vs PLI: $r_s = -0.47$, $P = 0.01$; EDA vs As: $r_s = -0.51$, $P = 0.01$; EDA vs Cu: $r_s = -0.42$, $P = 0.03$; EDA vs Hg: $r_s = -0.43$, $P = 0.02$; EDA vs Se: $r_s = -0.56$, $P < 0.001$; EDA vs Zn: $r_s = -0.55$, $P < 0.001$ and EDA vs PLI: $r_s = -0.62$, $P < 0.001$) (Table S6). Inversely, when looking at the influence of contaminants on individual fatty acids in the PF (Fig. 1D), it is possible to observe a higher dispersion of the FA throughout the biplot, indicating that metals are exerting a greater influence on the overall FA variability in this fraction. This is also visible from the more specific and highly consistent negative correlations between the metal levels and different FAs from the omega-3 group in the PF, also resulting in the negative correlation between the overall PLI and this omega-3 group ($r_s = -0.49$; $P = 0.01$) (Table S7). Given these results, more focus will be given to the PF fraction from here after.

The first two components of the polar biplot (Fig. 1C) accounted for 98.60 % of the variability in the data, where Cd, Cu, Se and the overlapping metals Fe, Al, Zn, and Mn showed the highest contribution to the dispersion of FAs classes. A negative influence of metals and metalloids on polyunsaturated fatty acids (PUFA) and highly unsaturated fatty

acids (HUFA) was observed. This negative correlation was significant for PUFA with As ($r_s = -0.43$; P = 0.03), Cu ($r_s = -0.57$; P < 0.001) and Se ($r_s = -0.41$; P = 0.03), and for HUFA with Fe ($r_s = -0.53$; P < 0.001) and with Se ($r_s = -0.56$; P < 0.001) (Table S7).

Interpreting the CCA with individual FAs in the polar fraction (Fig. 1D), the first two components of the biplot accounted for 52.90 % of the data variability, where Cd was the metal with higher influence in the FAs dispersion. The n3 group was the most affected by the metal contamination as seen by the dispersion in the CCA analysis (Fig. 1D) and by the several negative correlations found with the different elements for the four n3 FAs (18:3 n3 (linolenic acid, LNA), 20:3 n3 (eicosatrienoic acid (ETE)), 20:5 n3 (eicosapentaenoic acid, EPA), and 22:5 n3 (docosapentaenoic acid, DPA)), which even exhibited negative and significant correlations with the general PLI (Table S7). The LNA presented negative correlations with Al ($r_s = -0.40$; P = 0.04), As ($r_s = -0.44$; P = 0.02), Fe ($r_s = -0.46$; P = 0.02), Hg ($r_s = -0.39$; P = 0.04) and with Se ($r_s = -0.56$; P < 0.001), whereas ETE and EPA presented negative correlations with As ($r_s = -0.56$; P < 0.001; $r_s = -0.45$; P = 0.02), Cu ($r_s = -0.73$; P < 0.001; $r_s = -0.41$; P = 0.03) and Se ($r_s = -0.50$; P < 0.001; $r_s = -0.41$; P = 0.04), respectively (Table S7). DPA also presented negative correlations with As ($r_s = -0.53$; P < 0.001), Fe ($r_s = -0.55$; P < 0.001), Hg ($r_s = -0.46$; P = 0.02) and Se ($r_s = -0.71$; P < 0.001) (Table S7). The inverse was verified in the case of Pb, where positive and significant correlations were found between this metal's levels and ETE ($r_s = 0.52$; P = 0.01), EPA ($r_s = 0.45$; P = 0.02) and DPA ($r_s = 0.47$; P = 0.01). The n6 group did not seem to be as affected by the contaminant levels as the n3 group, as only 22:4 n6 FA correlated negatively with Mn ($r_s = -0.40$; P = 0.04), 22:2 n6 and 22:5 n6 FAs with Cd ($r_s = -0.39$; P = 0.04; $r_s = -0.39$; P = 0.04, respectively).

4. Discussion

The present study highlights the first reports about egg morphology, energy reserves and fatty acid profile, along with their relationship with contamination load by metals and metalloids in green sea turtles from the Eastern Atlantic region.

Compared to other oviparous reptiles, sea turtles, as non-annual breeders, experience heightened reproductive costs during the nesting season, involving migration, follicle production, and laying 2 to 5 clutches at two-week intervals, each comprising 50 to 150 eggs in most species. These efforts result in breeding intervals of 2 to 4 years (Le Gouvello et al., 2020; Miller, 1997). Thus, the scarce parental care of sea turtles is mainly translated in their energetic investment to increase their clutches and the number and/or size of their eggs (Le Gouvello et al., 2020).

The egg embryonic development starts afterwards, supported by nutrients accumulated in the egg, mostly through a myriad of lipids and proteins contained in the egg yolk (Hou and Fuiman, 2022). These provide energy for the metamorphosis and development of the early offspring. Maternal provisioning of nutrients to the egg, including essential fatty acids, is important for the normal development of the embryo and plays an important role in egg quality and, therefore, reproductive success and offspring performance (Hou and Fuiman, 2022; Carboni et al., 2013).

4.1. Comparative egg morphometry characteristics and lipid content

Sea turtle egg characteristics vary among species and are influenced by factors such as the species' reproductive biology and the environmental conditions of their nesting sites (Wallace et al., 2006). The egg diameter is a distinctive feature among species of sea turtles, reflecting their unique reproductive strategies and ecological adaptations where a better reproductive fitness translates into larger eggs and embryos (Sönmez, 2016; Topping and Valenzuela, 2021). Sea turtle eggshells share compositional and structural characteristics that are similar to those observed in other chelonians (Hirsch, 1983). The eggshells of green turtles contain various trace elements, including calcium, copper, potassium, sodium, strontium, and zinc (Jian et al., 2021). One important function of the eggshell is that it allocates approximately 60 % of its calcium content to support embryo development, while the egg content (yolk and albumen) supplies the remaining 40 % of the necessary calcium. This division of calcium resources supports the embryo's growth and development within the eggshell (Sahoo et al., 2009). One other important function is the respiratory gas exchange between the embryo and the nest environment (Ackerman, 1997).

Regarding egg morphometric measurements, present results indicate that egg diameter and whole egg weight of green sea turtles nesting in São Tomé Island are in general lower than the ones from the same species nesting in different regions of the Indian and Pacific Oceans (Chen and Cheng, 1995; Hanafy, 2012; Sinaei et al., 2018; Sönmez, 2016), but slightly higher in egg diameter compared with green sea turtles from Malaysia (Katni et al., 2022) (Table S8). Shell thickness of the eggs was also on average higher when compared to the results observed in green sea turtles from Thailand (Areekijseree et al., 2010) (Table S8). Despite the lack of information available for the remaining egg components in green sea turtles, the present values of yolk and albumin weight were in the same order of magnitude when compared with olive ridley turtles from India (Silas et al., 1984) (Table S8). The variations in egg characteristics observed in this study could be attributed to adaptations within the same species related to specific nesting environments and reproductive strategies. Differences in geographical locations and slight variations in diets unique to each zone may also contribute to variations in egg morphometrics and energy reserves (Jorgewich-Cohen et al., 2022). Additionally, distinct abiotic factors in these zones could potentially influence the characteristics of eggs within populations of the same species. The nest environments, including sand characteristics, nest temperatures, sand moisture, and nest location, vary among nesting beaches and are influenced by the prevailing climate type (Gravelle and Wyneken, 2022).

The neutral fraction [NF, consisting of triacylglycerols (TAG) and cholesteryl esters (CE)] serves as the primary energy source for embryo development, while the polar fraction [PF, comprising phospholipids (PL)] plays a vital structural role in cell membrane formation and signalling in tissue development in the growing embryo (Lawniczak and Teece, 2009). In the egg composition, the neutral or reserves fraction (NF) is considerably larger than the polar or metabolic fraction (PF). It is considered that the egg yolk has about 70 % of TAG, 22 % of PL, 1 % of ester cholesterol and 3–7 % of free cholesterol (Weng et al., 2020). Therefore, in terms of egg lipids, the present results showed lower values of MUFA and PUFA and higher SAT fatty acids values when compared with green sea turtles' eggs from Malaysia (Hashikin et al., 2021; Table S9).

Here, the NF demonstrated to be mainly composed of SAT and MUFA fatty acids and presented a different profile from the PF. During β-oxidation of FAs, the production of energy in form of Adenosine Triphosphate (ATP) uses preferably FAs with less unsaturation in their aliphatic chain, which is the case of palmitic and oleic acids (Dunning et al., 2014). Although SAT fatty acids showed similar levels in the two yolk fractions, the MUFA levels were higher in NF and especially due to the n9 group. As for the more unsaturated fatty acids, HUFA, the levels were lower in the NF, being consistent with the β-oxidation preference for reserve lipids (TAGs) with higher unsaturation levels.

4.2. Contaminant levels and genotoxic effects in females' blood

Following the work published previously on metals and metalloids levels in the blood of sea turtles (Morão et al., 2022), a general pollution load index (PLI) was here calculated for a more integrative overview of metal contamination data. To the best of our knowledge this index was applied here to sea turtles for the first time. According to the classification proposed by Goher et al. (2014), green sea turtles from this study, with an average PLI of 3.72, could be regarded as being strongly affected

by the metal contamination, although it must be noted that this classification index was developed for water contamination environments.

Genotoxicity biomarkers are used to evaluate and characterise damage endpoints to different contaminants in aquatic organisms (Canedo et al., 2021) such as sea turtles (Casini et al., 2018; Guevara-Meléndez et al., 2023; Labrada-Martagón et al., 2019). Here, ENAs assay was performed to look for possible genotoxic effects of metals and metalloids in green sea turtles. To date, basal frequency for micronuclei have been determined for freshwater turtles (3.56 ‰ ± 1.39; Latorre et al., 2015) and for lizards (0.95 ‰ ± 0.27; Schaumburg et al., 2012), but no baseline has been established for green sea turtles. The total ENAs values that we obtained were higher than those of green sea turtles from Yucatan Peninsula (18.56 ± 32.90 ‰) (Guevara-Meléndez et al., 2023), and this could suggest that the population of green sea turtles from São Tomé Island could be affected by environmental contamination from genotoxins. The correlation found between lobed nuclei and Cu and Hg levels (and overall PLI), could also indicate that the sea turtles from São Tomé Island are more exposed to metal contamination than the populations from the Mexican coast.

The study by Guevara-Meléndez et al. (2023) with juvenile green turtles found no association between body size, particularly curved carapace length (CCL), and the frequency of nuclear abnormalities. Conversely, da Silva et al. (2016) observed a positive correlation between the size of juvenile green turtles and the frequency of micronucleated cells with larger individuals presenting a higher frequency of these abnormalities. This could be indicative that older and larger specimens could be more susceptible to these erythrocytic abnormalities, such as the case of our report with adult females. In fact, also for other species of sea turtles (*Caretta caretta*) larger animals presented a higher ENAs frequency (Casini et al., 2018). This could be due to the longevity of these animals prolonging their exposure time to contamination and reducing DNA repair capabilities. However, although the female green turtles of the present work presented high ENAs frequencies, which could be an effect of age, it was also possible to observe an extra effect of metals on their erythrocytes, mainly due to the presence of Cu and Hg but also verified with the overall PLI (Table S4), with a higher probability of lobed nuclei to be formed. The lobed nuclei are considered precursors for MN formation in the cells and may represent a way to eliminate any amplified genetic material from the nucleus (Guevara-Meléndez et al., 2023; Mesak et al., 2019; Shimizu et al., 1998). Nirchio et al. (2019), observed in caimans, despite the different methodology used (e.g., comet assay), that Hg exposure had genotoxic effects on red blood cells. Hg exposure led to alterations in the erythrocyte physiology due to changes in the oxygen-binding capacity of hemoglobin and a decline in band 3-mediated ion exchange (Shalan, 2022); additionally, Hg can disrupt the antioxidant balance in erythrocytes, leading to increased lipid peroxidation and intensification of erythrocyte membrane lipid peroxidation processes causing deformations.

Concerning copper, the available literature indicates effects of this metal on erythrocytes, namely inducing the presence of micronuclei in fish (Kousar and Javed, 2015). A similar influence was observed in a study with amphibians, but with copper sulfate. In this case, exposure led to an increase in nuclear morphological abnormalities in bullfrog tadpoles, particularly the development of buds, lobes, and invaginations in the nuclei (da Rocha et al., 2012). In fact, copper ions can lead to significant changes in the structure of hemoglobin, oxidizing thiol groups in the globin chains of hemoglobin, resulting in the conversion of oxyhemoglobin to methemoglobin (Smith et al., 1993) which is unable to bind and transport oxygen effectively, leading to a decrease in the oxygen-carrying capacity (Ludlow et al., 2023).

In accordance with present results, micronuclei formation was also observed in skin fibroblasts of green sea turtles exposed to Cu and Hg (Finlayson et al., 2019), with the authors highlighting these and other metals as potential threats to sea turtles. The present results thus underscore the possible genotoxic effect of Cu and Hg on São Tomé Island green sea turtles.

As mentioned, age and metal exposure have been identified as contributing factors to nuclear abnormalities in green sea turtle erythrocytes. However, it is important to highlight that nuclear abnormalities can also be induced by other environmental contaminants, like persistent organic pollutants (POPs) not evaluated in this study. A study on loggerhead sea turtles, indicated a correlation between carcinogenic polycyclic aromatic hydrocarbons (PAHs) and comet assay results, suggesting that the observed DNA fragmentation levels could be attributed to these contaminants (Casini et al., 2018). This study demonstrates that our population from São Tomé Island could be exposed not only to heavy metal, but also to different classes of contaminants. Therefore, in the future, it would be important to understand which other contaminants may be accumulating in these turtles and also influencing the observed responses.

Other potential triggers for the development of nuclear abnormalities in reptiles include diseases, such as non-regenerative anemia (Stacy et al., 2011). In fact, nuclear alterations may also manifest in the erythrocytes of reptiles experiencing severe inflammatory conditions, malnutrition, starvation, or post-hibernation states which are related with anemia (Stacy et al., 2011). It was found that antineoplastic drugs are able to increase the number of abnormalities in aquatic turtles exposed to these compounds, assuming that the genotoxic damage could affect proteins or mechanisms responsible for forming microtubules, microfilaments, and/or intermediate filaments (Mesak et al., 2019). On the other hand, fibropapillomatosis (FP), a disease affecting all sea turtle species but especially the green sea turtles, is also considered a neoplastic disease (Monezi et al., 2016), which in turn have also been described to possibly be triggered by metals like Fe and Pb (da Silva et al., 2016). This underscores the complexity of conditions that can be involved in the appearance of nuclear abnormalities, but metal exposure seems to play a plausible role in it.

As mentioned above, these specific metals Hg and Cu could be having impacts on changes in the oxygen-binding capacity of hemoglobin with possible impacts at the organism level. However, the precise influence of lobed nuclei on erythrocyte function and their implications for overall organism require further research. Therefore, in future studies along with the analysis of more contaminants, it will be crucial to examine other cellular parameters indicating their health status which would be important to understand these impacts at higher biological levels.

4.3. Contaminant influence on egg morphometrics and yolk fatty acid profile

The reptile eggshell is essential for reproductive success, serving as a vital physiological structure for the embryo. It facilitates water and gas exchange, provides physical protection, and acts as a calcium reserve (Hallmann and Griebeler, 2015). As mentioned earlier, the eggshell allocates 60 % of its calcium to support embryonic development, and negative correlations have been found here among As and Cu and the shell thickness (Table S5). This could suggest that with an increase in these metals, the shell thickness becomes thinner, which could influence this calcium transfer during embryonic development and also result in more fragile protective structures.

Despite the available studies on the quantification of metals in the eggshells of sea turtles in the literature (Frossard et al., 2020), there are no studies mentioning the effects of such metal contamination in the eggshells in these animals. However, studies on birds indicate that eggshells have become thinner with anomalies in the microstructure in areas more exposed to metals (e.g., Mg, Cu, Zn, Pb, and Hg), suggesting a threat to the reproductive success of these animals (Rodriguez-Navarro et al., 2002). In this study, a similar phenomenon was observed with a thinner eggshell in individuals with higher As and Cu levels.

However, as mentioned before in the discussion, one cannot exclude that other contaminants may be also contributing for such results, like for instance dichlorodiphenyltrichloroethane (DDT) and

organochlorines (OCs) which have been shown to influence eggshell thickness in bird species (Lesch et al., 2024; Wiemeyer et al., 1984) and can potentially exert the same effect on turtle eggs given their similar chemical compositions (Gautron et al., 2021; Valdes et al., 2015). Nevertheless, the present observations suggest a lower egg quality, which may impact the reproductive success of these green turtles. This emphasizes the importance of continued investigation into the factors influencing egg quality in sea turtles.

The composition of fatty acids is known to be heavily influenced by the diet of organisms, often serving to distinguish between populations, developmental stages, or fitness levels (Filimonova et al., 2016). In this study, since all sampled individuals were adult females and strictly herbivorous at this life stage, consuming algae and seagrass (Jones and Seminoff, 2013), their diet signature is evidenced by the transference of long-chain PUFA to the egg for embryo formation (Craven et al., 2008). For instance, fatty acids like 18:2 n6 (ALA, alpha linoleic acid) and 18:3 n3 (LNA, linolenic acid) cannot generally be synthesized by animals and are therefore incorporated from the diet. Moreover, the marine seagrasses and algae are exposed to various contaminants like metals, which accumulate over time (Mourad and El-Azim, 2019; Solís et al., 2007) and can be transferred to green sea turtles through biomagnification processes (Ross et al., 2017). Therefore, taking into account the previous observations, it would be expected that increased metal levels acquired from the green turtle's diet would be accompanied by an increase in specific essential fatty acids from the algae and seagrass, such as LNA, ETE, EPA and DPA. Nonetheless, the results showed the opposite, with a negative correlation being observed between metals and the levels of those PUFA, suggesting an overall effect induced by metals.

During the nesting period, turtles abstain from feeding and rely on their accumulated reserves, which they metabolize for multiple nesting events throughout the breeding season (Gatto et al., 2020; Mortimer and Carr, 1987). It has been proposed that sea turtles undergo yolk deposition and follicular development before the breeding season, transferring their lipids and fatty acid (FAs) signatures to the eggs. This process aims to ensure consistent lipid content in yolks across clutches (Hamann et al., 2002). As major components of most lipids, FAs play a functional role as a source of metabolic energy (NF) and as structural components (PF) for development and as precursors of bioactive molecules (Filimonova et al., 2016; Carboni et al., 2013). Polar lipids are also a source of phosphate for nucleic acid synthesis and choline for neurotransmission (Hou and Fuiman, 2022).

The literature demonstrates that metallic elements have a high affinity to biological structures and molecules such as FAs, thus interacting indirectly (through enzymatic interaction and Reactive Oxygen Species-ROS production) with the FA metabolism at the cellular level and changing their composition in the organisms of the aquatic environment (Filimonova et al., 2016). Such changes are often detrimental to living compartments, manifested mainly through lipidic oxidative events in membranes rather than in lipidic reserves, with consequent disruption of structural and physicochemical properties of membranes (Filimonova et al., 2016; Silva et al., 2017). The present results showed more correlations between metals and the polar phospholipidic fractions of yolk fatty acids, where mostly FAs with higher degrees of unsaturation, and essential in embryogenesis (PUFA and HUFA), correlated negatively with the elements analysed. This is in accordance with the literature showing that FAs with higher unsaturation are more readily oxidized (Ayala et al., 2014). Additionally, fatty acids also act as response tools to environmental stressors, and it is known that in the presence of contamination, membranes become more rigid and impermeable indicating that fatty acids with less unsaturation (SAT and MUFA) will be more present in these structures (Filimonova et al., 2016). Again, in this study several positive correlations were observed between SAT fatty acids and the elements analysed, suggesting that the metals' effects in reproductive females could be compromising the quality of their eggs mostly through phospholipids. Therefore, the discussion will emphasise the FA profiling obtained in the polar fraction and their relation to contaminant load.

As components of cell membrane phospholipids, Long-Chain FAs serve as precursors of eicosanoids, and act as ligands for membrane receptors and transcription factors that regulate gene expression (Izquierdo and Koven, 2011), being thus necessary to ensure proper reproduction (i.e., oocyte maturation, ovulation, nesting and fertilization) and embryo development, as demonstrated in several species, both marine and terrestrial (Callet et al., 2022). In the present study, the n3 LNA, ETE, EPA and DPA were the FAs that more negatively correlated individually with the metals analysed. This was further evidenced by the correlations with the global influence of the contaminants (PLI, Table S7).

LNA correlated negatively with the essential metals Cu, Fe and Se, and the non-essential As and Hg, which is highly indicative that this FA was particularly affected by the contaminant load in female green sea turtles. Despite more abundant for terrestrial vertebrates, there is literature elucidating the benefits of LNA in early embryonic development and its role in promoting reproductive success. LNA proves to be an essential fatty acid in larvae development, primarily serving as a precursor of HUFAs such as EPA and DHA through elongase activity. This was advanced by Martins et al. (2020), who substantiated the significance of this fatty acid during the early developmental stages of *Prochilodus argenteus* fish. Also, it has been demonstrated in common carp (*Cyprinus carpio*) that higher dietary LNA/LA ratios alter both growth and ovarian development (Ma et al., 2020) and in yellow catfish (*Pelteobagrus fulvidraco*) this can promote gonadotropins synthesis during ovarian development (Fei et al., 2020), thus promoting reproductive success. Additionally, it was demonstrated that LNA promoted embryo development, allegedly by its ability to increase active MAPK signalling (Marei et al., 2010) in bovine ovaries. All this suggests that the decrease in LNA levels with the increase of metal contamination in the present sea turtles, may eventually compromise the normal development of the embryo in those sea turtle eggs.

ETE correlated negatively with As, Cu, Hg, Se, and Zn. This particular essential FA stands out as one of the most actively engaged in regulating the elongation and desaturation reactions that convert dietary C-18 fatty acids to C-20 eicosanoid precursors (Holman, 1986). The eicosanoids are highly bioactive lipids, entangled to a variety of physiological functions, including reproduction through both autocrine and paracrine signalling (Gil and Fontana, 2022), and more particularly in extraembryonic membranes such as the chorioallantoic membrane, during embryonic development in oviparous species (Cantu et al., 2016). As for EPA, negative correlations were obtained with As, and the essential elements Cu and Se. In the same way to ETE, EPA alongside with LNA are also strictly involved in vital eicosanoid synthesis like prostaglandins and leukotrienes (Marei et al., 2009). These eicosanoids play roles in key processes such as final maturation, ovulation, and spawning, as documented for sea bass (Sorbera et al., 2001). In sea turtles, mostly prostaglandin eicosanoids are enrolled to reproductive development (Blanvillain et al., 2010), and thus tangled to the mentioned fatty acids. With the accumulation of the elements mentioned above and the availability of ETE, EPA and LNA, the eicosanoid pathway may be disrupted and consequently the normal reproductive development. Moreover, HUFA like EPA are documented to be linked to reproduction success as key components of cell membranes and ensure their correct physiological functions, covering vital stages like oocyte maturation, ovulation, spawning, fertilization, and embryonic development, evident across diverse fish species (Callet et al., 2022; Tocher, 2003). EPA is also known for its anti-inflammatory properties, involvement in cell membrane structure, and support for neural and visual development (Callet et al., 2022).

Comparatively to other essential n3 HUFA fatty acids such as EPA and DHA, DPA is a poorly understood fatty acid in the literature and it was found in relatively high amounts in egg yolk of the present turtles, comparatively. DPA possess the putative capacity to be converted into

DHA and retro-converted into EPA (Drouin et al., 2019). Burns and Fuiman (2019) correlated DPA levels in eggs of southern flounder (*Paralichthys lethostigma*) to a higher concentration of DHA in their larvae stage, relating this to increased predator evasion abilities. Also, this understudied FA possesses 10-fold greater endothelial cell migration ability than EPA, essential during early embryo vascular development (Burns and Fuiman, 2019).

Due to their importance and the overall correlations with the metals analysed and the PLI index, these LNA, ETE, EPA, and DPA fatty acids seem to be the most appropriate to be considered as proxies for potential effects of metal contamination in the success of embryo development of green sea turtles and possibly of other oviparous organisms.

5. Conclusion

To the best of knowledge, this study represents a first attempt to explore how contamination by several metal elements in reproductive female sea turtles may influence the quality of their eggs in terms of their structure and lipidic composition, giving some indications of possible consequences for their reproductive and developmental success. It is also the first morphometric characterisation and lipidic profile of green sea turtles' eggs from the Gulf of Guinea in Eastern Atlantic region.

By characterising the overall metal load (PLI) of the sampled individuals, green sea turtles from the region could be regarded as being exposed to moderate to high-risk metal contamination. Those metal levels in the blood of reproductive females were shown here to be associated with genotoxic effects in their erythrocytes (mainly Cu and Hg but also overall PLI), along with a decrease in eggshell thickness (mainly Cu and As) and alterations in the fatty acid profile of their eggs (including PLI). This FA profile alteration was observed for both egg yolk fractions, although the effects were more evident in the metabolic fraction (PF) where a higher number of significant correlations (and stronger) were observed between metal and FA levels. In this fraction, the n3 FA group was the most negatively affected by the levels of metals. As mentioned, the accumulation of metals and the anticipated reduction in the availability of ETE, EPA, LNA, and DPA may disrupt the eicosanoid and other crucial pathways, thereby affecting normal reproductive development.

The nesting population of green sea turtles in the São Tomé and Principe archipelago is acknowledged as a genetically distinct rookery. The data analysed here demonstrated that embryonic development in sea turtles from São Tomé may be impaired by the increasing metal levels that they bioaccumulate, raising concern about the future generations of these populations. This information is vital not only for understanding the potential impacts of metal contamination over generations but also to increase valuable information for stakeholder to draw the tools for setting policies and conservation initiatives for the sea turtles and their habitats.

CRediT authorship contribution statement

Inês F.C. Morão: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Tiago Simões:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Roger B. Casado:** Methodology, Investigation. **Sara Vieira:** Writing – review & editing, Investigation. **Betânia Ferreira-Airaud:** Writing – review & editing, Investigation. **Ilaria Caliani:** Writing – review & editing, Methodology, Investigation. **Agata Di Noi:** Writing – review & editing, Methodology, Investigation. **Silvia Casini:** Writing – review & editing, Methodology. **Maria C. Fossi:** Writing – review & editing, Resources. **Marco F.L. Lemos:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Sara C. Novais:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.172710.

References

Ackerman, R.A., 1997. The nest environment and the embryonic development of sea turtles. In: The Biology of Sea Turtles, vol. 1. CRC Press, Boca Raton, pp. 83–106.

Alves, L.M.F., Nunes, M., Marchand, P., Le Bizec, B., Mendes, S., Correia, J.P.S., Lemos, M.F.L., Novais, S.C., 2016. Blue sharks (*Prionace glauca*) as bioindicators of pollution and health in the Atlantic Ocean: contamination levels and biochemical stress responses. Sci. Total Environ. 563–564, 282–292. https://doi.org/10.1016/j.scitotenv.2016.04.085.

Alves, L.M.F., Lemos, M.F.L., Moutinho, A.B., Ceia, F.R., Muñoz-Arnanz, J., Jiménez, B., Cabral, H., Novais, S.C., 2023. Assessment of contaminants in blue sharks from the Northeast Atlantic: profiles, accumulation dynamics, and risks for human consumers. Environ. Pollut. 316, 120467 https://doi.org/10.1016/J.ENVPOL.2022.120467.

Angulo, E., 1996. The Tomlinson Pollution Load Index applied to heavy metal, "mussel-watch" data: a useful index to assess coastal pollution. Sci. Total Environ. 187 (1), 19–56. https://doi.org/10.1016/0048-9697(96)05128-5.

Areekijseree, M., Nuamsukon, S., Chuen-Im, T., Narkkong, N.-A., 2010. Microanalysis on ultrastructure and elemental composition of green turtle (*Chelonia mydas*) eggshells. J. Microsc. Soc. Thailand 24 (2), 78–82.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Med. Cell. Longev. 2014, 360438 https://doi.org/10.1155/2014/360438.

Barraza, A.D., Finlayson, K.A., Leusch, F.D.L., van de Merwe, J.P., 2021. Systematic review of reptile reproductive toxicology to inform future research directions on endangered or threatened species, such as sea turtles. Environ. Pollut. (Barking, Essex: 1987) 286, 117470. https://doi.org/10.1016/j.envpol.2021.117470.

Blanvillain, G., Owens, D.W., Kuchling, G., 2010. Hormones and reproductive cycles in turtles. In: Hormones and Reproduction of Vertebrates: Reptiles. Elsevier, pp. 277–303. https://doi.org/10.1016/B978-0-12-374930-7.10010-X.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37 (8), 911–917. https://doi.org/10.1139/O59-099.

Burns, C.M., Fuiman, L.A., 2019. Maternally derived nutrients influence fatty acid composition and predator evasion behavior of larval southern flounder, *Paralichthys lethostigma*. J. Exp. Mar. Biol. Ecol. 514–515, 41–47. https://doi.org/10.1016/j.jembe.2019.03.008.

Callet, T., Cardona, E., Turonnet, N., Maunas, P., Larroquet, L., Surget, A., Corraze, G., Panserat, S., Marandel, L., 2022. Alteration of eggs biochemical composition and progeny survival by maternal high carbohydrate nutrition in a teleost fish. Sci. Rep. 12 (1) https://doi.org/10.1038/s41598-022-21185-5.

Canedo, A., de Jesus, L.W.O., Bailão, E.F.L.C., Rocha, T.L., 2021. Micronucleus test and nuclear abnormality assay in zebrafish (*Danio rerio*): past, present, and future trends. Environ. Pollut. 290, 118019 https://doi.org/10.1016/J.ENVPOL.2021.118019.

Cantu, T.M., Bowden, J.A., Scott, J., Pérez-Viscasillas, J.B., Huncik, K., Guillette, M.P., Guillette, L.J., 2016. Alterations in eicosanoid composition during embryonic development in the chorioallantoic membrane of the American alligator (*Alligator*

mississippiensis) and domestic chicken (*Gallus gallus*). Gen. Comp. Endocrinol. 238, 78–87. https://doi.org/10.1016/j.ygcen.2016.07.006.
Carboni, S., Hughes, A.D., Atack, T., Tocher, D.R., Migaud, H., 2013. Fatty acid profiles during gametogenesis in sea urchin (*Paracentrotus lividus*): effects of dietary inputs on gonad, egg and embryo profiles. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 164 (2), 376–382. https://doi.org/10.1016/j.cbpa.2012.11.010.
Carr, A., 1982. Notes on the behavioral ecology of sea turtles. In: Bjorndal, K. (Ed.), Biology and Conservation of Sea Turtles. Smithsonian Institution Press, pp. 19–26.
Casini, S., Caliani, I., Giannetti, M., Marsili, L., Maltese, S., Coppola, D., Bianchi, N., Campani, T., Ancora, S., Caruso, C., Furii, G., Parga, M., D'Agostino, A., Fossi, M.C., 2018. First ecotoxicological assessment of *Caretta caretta* (Linnaeus, 1758) in the Mediterranean Sea using an integrated nondestructive protocol. Sci. Total Environ. 631–632, 1221–1233. https://doi.org/10.1016/j.scitotenv.2018.03.111.
Chen, H., Cheng, J., 1995. Breeding biology of the green turtle, *Chelonia mydas*, (Reptilia: Cheloniidae) on Wan-An Island, Peng-Hu Archipelago, Taiwan. I. Nesting Ecology. Mar. Biol. 124, 9–15.
Cortés-Gómez, A.A., Morcillo, P., Guardiola, F.A., Espinosa, C., Esteban, M.A., Cuesta, A., Girondot, M., Romero, D., 2018. 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.
Craven, K.S., Parsons, J., Taylor, S.A., Belcher, C.N., Owens, D.W., 2008. The influence of diet on fatty acids in the egg yolk of green sea turtles, *Chelonia mydas*. J. Comp. Physiol. B: Biochem. Syst. Environ. Physiol. 178 (4), 495–500. https://doi.org/10.1007/s00360-007-0242-8.
da Rocha, C.A.M., Almeida, V.H. da C., Costa, A. da S., Sagica-Júnior, J.C., Monteiro, J.A. N., de Souza, Y.S.R., da Cunha, L.A., Pinheiro, R.H. da S., 2012. Induction of micronuclei and other nuclear abnormalities in bullfrog tadpoles (*Lithobates catesbeianus*) treated with copper sulphate. Int. J. Genet. 2 (1), 6–11. https://doi.org/10.5829/idosi.ijg.2012.2.1.61179.
da 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.
Donato, P., Dugo, P., Mondello, L., 2017. Separation of lipids. In: Liquid Chromatography: Applications: Second Edition, , second ed.vol. 2. Elsevier Inc. https://doi.org/10.1016/B978-0-12-805392-8.00008-6
Drouin, G., Catheline, D., Guillocheau, E., Gueret, P., Baudry, C., Le Ruyet, P., Rioux, V., Legrand, P., 2019. Comparative effects of dietary n-3 docosapentaenoic acid (DPA), DHA and EPA on plasma lipid parameters, oxidative status and fatty acid tissue composition. J. Nutr. Biochem. 63, 186–196. https://doi.org/10.1016/j.jnutbio.2018.09.029.
Duarte, B., Feijão, E., Franzitta, M., Duarte, I.A., de Carvalho, R.C., Cabrita, M.T., Marques, J.C., Caçador, I., Fonseca, V., Matos, A.R., 2022. LipidTOX: A fatty acid-based index efficient for ecotoxicological studies with marine model diatoms exposed to legacy and emerging contaminants. Ecol. Indic. 139 https://doi.org/10.1016/j.ecolind.2022.108885 (1470–160).
Dunning, K.R., Russell, D.L., Robker, R.L., 2014. Lipids and oocyte developmental competence: the role of fatty acids and β-oxidation. In: Reproduction, vol. 148, (1). Bioscientifica Ltd., pp. R15–R27. https://doi.org/10.1530/REP-13-0251
Evans, J.D., 1996. Straightforward Statistics for the Behavioral Sciences. Brooks/Cole Publishing, Pacific Grove, Calif.
Fei, S., Liu, C., Xia, Y., Liu, H., Han, D., Jin, J., Yang, Y., Zhu, X., Xie, S., 2020. The effects of dietary linolenic acid to linoleic acid ratio on growth performance, tissues fatty acid profile and sex steroid hormone synthesis of yellow catfish *Pelteobagrus fulvidraco*. Aquac. Rep. 17 https://doi.org/10.1016/j.aqrep.2020.100361.
Ferreira-Airaud, B., Schmitt, V., Vieira, S., do Rio, M.J. de C., Neto, E., Pereira, J., 2022. The sea turtles of São Tomé and Príncipe: diversity, distribution, and conservation status. In: Ceríaco, L.M.P., de Lima, R.F., Melo, M., Rayna, B.C. (Eds.), Biodiversity of the Gulf of Guinea Oceanic Islands. Science and Conservation. Springer, pp. 535–554. https://doi.org/10.1007/978-3-031-06153-0.
Filimonova, V., Gonçalves, F., Marques, J.C., De Troch, M., Gonçalves, A.M.M., 2016. Fatty acid profiling as bioindicator of chemical stress in marine organisms: a review. Ecol. Indic. 67, 657–672. https://doi.org/10.1016/j.ecolind.2016.03.044.
Finlayson, K.A., Leusch, F.D.L., van de Merwe, J.P., 2019. Primary green turtle (*Chelonia mydas*) skin fibroblasts as an in vitro model for assessing genotoxicity and oxidative stress. Aquat. Toxicol. 207, 13–18. https://doi.org/10.1016/j.aquatox.2018.11.022.
Fokina, N.N., Ruokolainen, T.R., Nemova, N.N., Bakhmet, I.N., 2013. Changes of blue mussels *Mytilus edulis* L. lipid composition under cadmium and copper toxic effect. Biol. Trace Elem. Res. 154 (2), 217–225. https://doi.org/10.1007/S12011-013-9727-3.
Formia, A., Godley, B.J., Dontaine, J.F., Bruford, M.W., 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conserv. Genet. 7 (3), 353–369. https://doi.org/10.1007/s10592-005-9047-z.
Frossard, A., Vieira, L.V., Carneiro, M.T.W.D., Gomes, L.C., Chippari-Gomes, A.R., 2020. Accumulation of trace metals in eggs and hatchlings of *Chelonia mydas*. J. Trace Elem. Med. Biol. 62, 126654 https://doi.org/10.1016/j.jtemb.2020.126654.
Fuchs, B., Süß, R., Teuber, K., Eibisch, M., Schiller, J., 2011. Lipid analysis by thin-layer chromatography-a review of the current state. J. Chromatogr. A 1218 (19), 2754–2774. https://doi.org/10.1016/j.chroma.2010.11.066.
Gatto, C.R., Robinson, N.J., Spotila, J.R., Paladino, F.V., Santidrián Tomillo, P., 2020. Body size constrains maternal investment in a small sea turtle species. Mar. Biol. 167 (12), 1–11. https://doi.org/10.1007/s00227-020-03795-7.
Gautron, J., Stapane, L., Le Roy, N., Nys, Y., Rodriguez-Navarro, A.B., Hincke, M.T., 2021. Avian eggshell biomineralization: an update on its structure, mineralogy and protein tool kit. BMC Mol. Cell Biol. 22 (1), 1–17. https://doi.org/10.1186/s12860-021-00350-0.
Gil, Á., Fontana, L., 2022. Eicosanoids: prostaglandins, leukotrienes, thromboxanes and related compounds. In: Reference Module in Food Science. https://doi.org/10.1016/B978-0-12-821848-8.00177-3.
Godfrey, M.H., Godley, B.J., 2008. Seeing past the red: flawed IUCN global listings for sea turtles. Endanger. Species Res. 6 (2), 155–159. https://doi.org/10.3354/esr00071.
Goher, M.E., Hassan, A.M., Abdel-Moniem, I.A., Fahmy, A.H., El-Sayed, S.M., 2014. Evaluation of surface water quality and heavy metal indices of Ismailia Canal, Nile River, Egypt. Egypt. J. Aquat. Res. 40 (3), 225–233. https://doi.org/10.1016/j.ejar.2014.09.001.
Gonçalves, A.M.M., Marques, J.C., Gonçalves, F., Gonçalves, A.M.M., Marques, J.C., Gonçalves, F., 2017. Fatty acids' profiles of aquatic organisms: revealing the impacts of environmental and anthropogenic stressors. In: Fatty Acids. https://doi.org/10.5772/INTECHOPEN.68544.
Gravelle, J., Wyneken, J., 2022. Resilient eggs: highly successful loggerhead sea turtle nesting sites vary in their characteristics. Front. Ecol. Evol. 10 (April), 1–14. https://doi.org/10.3389/fevo.2022.853835.
Guevara-Meléndez, A.M., Comas-Garcia, M., Labrada-Martagón, V., 2023. Description and quantification of micronucleus and nuclear abnormalities in erythrocytes of the sentinel green turtle (*Chelonia mydas*) with fluorescence microscopy. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 887 https://doi.org/10.1016/j.mrgentox.2023.503596.
Guirlet, E., Das, K., Girondot, M., 2008. Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. Aquat. Toxicol. 88 (4), 267–276. https://doi.org/10.1016/j.aquatox.2008.05.004.
Guo, X., 2014. Advances in Gas Chromatography. InTechOpen. https://doi.org/10.5772/57016.
Hallmann, K., Griebeler, E.M., 2015. Eggshell types and their evolutionary correlation with life-history strategies in squamates. PLoS ONE 10 (9). https://doi.org/10.1371/journal.pone.0138785 (PLOS).
Hamann, M., Limpus, C.J., Whittier, J.M., 2002. Patterns of lipid storage and mobilisation in the female green sea turtle *Chelonia mydas*. J. Comp. Physiol. B: Biochem. Syst. Environ. Physiol. 172 (6), 485–493. https://doi.org/10.1007/s00360-002-0271-2.
Hanafy, M., 2012. Nesting of marine turtles on the Egyptian beaches of the Red Sea. Egypt. J. Aquat. Biol. Fish. 16 (2), 59–71. https://doi.org/10.21608/ejabf.2012.2125.
Hancock, J.M., Vieira, S., Taraveira, L., Santos, A., Schmitt, V., Semedo, A., Patrício, A. R., Ferrand, N., Gonçalves, H., Sequeira, F., 2019. Genetic characterization of green turtles (*Chelonia mydas*) from São Tomé and Príncipe: insights on species recruitment and dispersal in the Gulf of Guinea. J. Exp. Mar. Biol. Ecol. 518 (June), 151181 https://doi.org/10.1016/j.jembe.2019.151181.
Hashikin, N.K., Amirul, F.M.A., Affan, A.A., Ghani, A.A.A., Rinalfi, T.P.T.A., Zakaria, Z., Azlan, C.A., Hassim, H.A., 2021. Comparative study on nutritional compositions and pathogenic microorganism isolated from green turtle egg and chicken egg. J. Food Nutr. Res. 9 (3), 142–147. https://doi.org/10.12691/jfnr-9-3-6.
Hewavisenthi, S., Parmenter, C.J., 2002. Egg components and utilization of yolk lipids during development of the flatback turtle *Natator depressus*. J. Herpetol. 36 (1), 43–50. https://doi.org/10.1670/0022-1511(2002)036[0043:ECAUOY]2.0.CO;2.
Hirsch, K.F., 1983. Contemporary and fossil chelonian eggshells. Copeia 1983 (2), 382. https://doi.org/10.2307/1444381.
Holman, R.T., 1986. Control of polyunsaturated acids in tissue lipids. J. Am. Coll. Nutr. 5 (2), 183–211. https://doi.org/10.1080/07315724.1986.10720125.
Hou, Z., Fuiman, L.A., 2022. Incorporation of dietary lipids and fatty acids into red drum *Sciaenops ocellatus* eggs. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 258 https://doi.org/10.1016/j.cbpb.2021.110694.
Hsu, L.L., Culhane, A.C., 2020. Impact of data preprocessing on integrative matrix factorization of single cell data. Front. Oncol. 10, 973. https://doi.org/10.3389/fonc.2020.00973.
IBM SPSS Statistics 28.0.1.0. IBM Analytics, 2023. https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-28010.
Izquierdo, M.S., Koven, W., 2011. Lipids in larval fish nutrition. In: Larval Fish Nutrition, pp. 47–82.
Jian, L., Guo, R., Zheng, X., Shi, H., Wang, J., 2021. Trace elements in green turtle eggshells and coral sand sediments from the Xisha Islands, South China Sea. Mar. Pollut. Bull. 164, 112036 https://doi.org/10.1016/j.marpolbul.2021.112036.
Johnson, M., Finlayson, K., Shelper, T., van de Merwe, J.P., Leusch, F.D.L., 2022. Optimisation of an automated high-throughput micronucleus (HiTMiN) assay to measure genotoxicity of environmental contaminants. Chemosphere 298, 134349. https://doi.org/10.1016/j.chemosphere.2022.134349.
Jones, T.T., Seminoff, J.A., 2013. Feeding biology advances from field-based observations, physiological studies, and molecular techniques. In: Wyneken, J., Lohmann, K.J., Musick, J.A. (Eds.), The Biology of Sea Turtles Vol iii, vol. III. CRC Press, pp. 211–248.
Jorgewich-Cohen, G., Henrique, R.S., Dias, P.H., Sánchez-Villagra, M.R., 2022. The evolution of reproductive strategies in turtles. PeerJ 10, 1–16. https://doi.org/10.7717/peerj.13014.
Katni, N.H., Azmi, A.F.M., Abdullah, M.M., Rusli, M.U., Zakaria, Z., Azizan, T.R.P.T., Amat, A.C., Saad, M.Z., Yasin, I.S.M., Nazarudin, M.F., Hassim, H.A., 2022. Nutritional compositions, pathogenic microorganisms and heavy metal concentration in green turtle eggs (*Chelonia mydas*) from Terengganu and Sabah, Malaysia. Front. Mar. Sci. 9, 1643. https://doi.org/10.3389/fmars.2022.948427.
Kaur, N., Brraich, O.S., 2022. Impact of industrial effluents on physico-chemical parameters of water and fatty acid profile of fish, *Labeo rohita* (Hamilton), collected

from the Ramsar sites of Punjab, India. Environ. Sci. Pollut. Res. 29 (8), 11534–11552. https://doi.org/10.1007/s11356-021-16429-2.
Kousar, S., Javed, M., 2015. Studies on induction of nuclear abnormalities in peripheral blood erythrocytes of fish exposed to copper. Turk. J. Fish. Aquat. Sci. 15 (4), 870–886. https://doi.org/10.4194/1303-2712-v15_4_11.
Labrada-Martagón, V., Teneriá, F.A.M., Zenteno-Savín, T., 2019. Standardized micronucleus assay for peripheral blood from sea turtles. Chelonian Conserv. Biol. 18 (2), 175–186. https://doi.org/10.2744/CCB-1373.1.
Latorre, M.A., González, E.C.L., Siroski, P.A., Poletta, G.L., 2015. Basal frequency of micronuclei and hematological parameters in the side-necked turtle, *Phrynops hilarii* (Duméril & Bibron, 1835). Acta Herpetol. 10 (1), 31–37. https://doi.org/10.13128/Acta.
Lawniczak, C.J., Teece, M.A., 2009. Lipid metabolism during embryonic development of the common snapping turtle, *Chelydra serpentina*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 153 (1), 73–80. https://doi.org/10.1016/j.cbpb.2009.01.016.
Le Gouvello, D.Z.M., Nel, R., Cloete, A.E., 2020. The influence of individual size on clutch size and hatchling fitness traits in sea turtles. J. Exp. Mar. Biol. Ecol. 527, 151372 https://doi.org/10.1016/j.jembe.2020.151372.
Lepš, J., Šmilauer, P., 2003. Multivariate analysis of ecological data using CANOCO. In: Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press. https://doi.org/10.1017/cbo9780511615146.
Lesch, V., Kylin, H., Bouwman, H., 2024. The Cattle Egret *Bubulcus ibis* as a near-global indicator of terrestrial pollution. Environ. Chem. Ecotoxicol. 6, 15–25. https://doi.org/10.1016/j.enceco.2023.11.003.
López-Berenguer, G., Peñalver, J., Martínez-López, E., 2020. A critical review about neurotoxic effects in marine mammals of mercury and other trace elements. Chemosphere 246, 125688. https://doi.org/10.1016/J.CHEMOSPHERE.2019.125688.
Ludlow, J.T., Wilkerson, R.G., Nappe, T.M., 2023. Methemoglobinemia. In: StatPearls. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK537317/.
Ma, X., Wang, L., Xie, D., Tian, X., Zhang, Y., Wu, L., Liu, H., Wang, L., Dong, C., Li, X., Nie, G., 2020. Effect of dietary linolenic/linoleic acid ratios on growth performance, ovarian steroidogenesis, plasma sex steroid hormone, and tissue fatty acid accumulation in juvenile common carp, *Cyprinus carpio*. Aquac. Rep. 18 https://doi.org/10.1016/j.aqrep.2020.100452.
Mach, M.E., Martone, R.G., Chan, K.M.A., 2015. Human impacts and ecosystem services: insufficient research for trade-off evaluation. Ecosyst. Serv. J. 16, 112–120. https://doi.org/10.1016/j.ecoser.2015.10.018.
Marei, W.F., Wathes, D.C., Fouladi-Nashta, A.A., 2009. The effect of linolenic acid on bovine oocyte maturation and development. Biol. Reprod. 81 (6), 1064–1072. https://doi.org/10.1095/biolreprod.109.076851.
Marei, W.F., Wathes, D.C., Fouladi-Nashta, A.A., 2010. Impact of linoleic acid on bovine oocyte maturation and embryo development. Reproduction 139 (6), 979–988. https://doi.org/10.1530/REP-09-0503.
Martins, E. de F.F., Magnone, L., Bessonart, M., Costa, D.C., dos Santos, J.C.E., Bazolli, N., Nakayama, C.L., Luz, R.K., 2020. Lipid content and fatty acid dynamics of female muscle, oocytes and larvae of *Prochilodus argenteus* (Spix & Agassiz, 1829). Aquac. Rep. 17 https://doi.org/10.1016/j.aqrep.2020.100377.
McKeegan, P.J., Sturmey, R.G., 2011. The role of fatty acids in oocyte and early embryo development. Reprod. Fertil. Dev. 24 (1), 59–67. https://doi.org/10.1071/RD11907.
Mesak, C., Montalvão, M.F., Paixão, C.F.C., Mendes, B. de O., Araújo, A.P. da C., Quintão, T.C., Malafaia, G., 2019. Do Amazon turtles exposed to environmental concentrations of the antineoplastic drug cyclophosphamide present mutagenic damages? If so, would such damages be reversible? Environ. Sci. Pollut. Res. 26 (6), 6234–6243. https://doi.org/10.1007/s11356-019-04155-9.
Miller, J.D., 1997. Reproduction in sea turtles. In: Lutz, P.L., Musick, J.A. (Eds.), The Biology of Sea Turtles, vol. I. CRC Press, pp. 51–81. https://doi.org/10.1201/9780203737088.
Monezi, T.A., Mehnert, D.U., de Moura, E.M.M., Muller, N.M.G., Garrafa, P., Matushima, E.R., Werneck, M.R., Borella, M.I., 2016. Chelonid herpesvirus 5 in secretions and tumor tissues from green turtles (*Chelonia mydas*) from Southeastern Brazil: a ten-year study. Vet. Microbiol. 186, 150–156. https://doi.org/10.1016/j.vetmic.2016.02.020.
Morão, I.F.C., Lemos, M.F.L., Félix, R., Vieira, S., Barata, C., Novais, S.C., 2022. Stress response markers in the blood of São Tomé green sea turtles (*Chelonia mydas*) and their relation with accumulated metal levels. Environ. Pollut. 293, 118490 https://doi.org/10.1016/j.envpol.2021.118490.
Morcillo, P., Esteban, M., Cuesta, A., 2016. Heavy metals produce toxicity, oxidative stress and apoptosis in the marine teleost fish SAF-1 cell line. Chemosphere 144, 225–233. https://doi.org/10.1016/j.chemosphere.2015.08.020.
Mortimer, J.A., Carr, A., 1987. Reproduction and migrations of the Ascension Island green turtle (*Chelonia mydas*). Copeia 1, 103–113. https://doi.org/10.2307/1446043.
Mourad, F.A., El-Azim, H.A., 2019. Use of green alga *Ulva lactuca* (L.) as an indicator to heavy metal pollution at intertidal waters in Suez gulf, Aqaba gulf and Suez canal, Egypt. Egypt. J. Aquat. Biol. Fish. 23 (4), 437–449. https://doi.org/10.21608/ejabf.2019.57913.
Nirchio, M., Choco-Veintimilla, O., Quizhpe-Cordero, P.F., Hernández, J.G., Oliveira, C., 2019. Genotoxic effects of mercury chloride on the neotropical fish *Andinoacara rivulatus* (Cichlidae: Cichlasomatini). Rev. Biol. Trop. 67 (4), 745–754. https://doi.org/10.15517/rbt.v67i4.34133.
Páez-Osuna, F., Calderón-Campuzano, M.F., Soto-Jiménez, M.F., Ruelas-Inzunza, J.R., 2010. Trace metals (Cd, Cu, Ni, and Zn) in blood and eggs of the sea turtle *Lepidochelys olivacea* from a nesting colony of Oaxaca, Mexico. Arch. Environ. Contam. Toxicol. 59 (4), 632–641. https://doi.org/10.1007/s00244-010-9516-3.
Páez-Osuna, F., Calderón-Campuzano, M.F., Soto-Jiménez, M.F., Ruelas-Inzunza, J., 2011. Mercury in blood and eggs of the sea turtle *Lepidochelys olivacea* from a nesting colony in Oaxaca, Mexico. Mar. Pollut. Bull. 62 (6), 1320–1323. https://doi.org/10.1016/j.marpolbul.2011.04.004.
Perrault, J., Wyneken, J., Thompson, L.J., Johnson, C., Miller, D.L., 2011. Why are hatching and emergence success low? Mercury and selenium concentrations in nesting leatherback sea turtles (*Dermochelys coriacea*) and their young in Florida. Mar. Pollut. Bull. 62 (8), 1671–1682. https://doi.org/10.1016/j.marpolbul.2011.06.009.
Rodriguez-Navarro, A.B., Gaines, K.F., Romanek, C.S., Masson, G.R., 2002. Mineralization of clapper rail eggshell from a contaminated salt marsh system. Arch. Environ. Contam. Toxicol. 43 (4), 449–460. https://doi.org/10.1007/s00244-002-0266-8.
Ross, D.A.N., Guzmán, H.M., Potvin, C., van Hinsberg, V.J., 2017. A review of toxic metal contamination in marine turtle tissues and its implications for human health. Reg. Stud. Mar. Sci. 15, 1–9. https://doi.org/10.1016/j.rsma.2017.06.003.
Sahoo, G., Mohapatra, B.K., Dutta, S.K., 2009. Structural changes in olive ridley turtle eggshells during embryonic development. Herpetol. J. 19 (3), 143–149.
Schaumburg, L.G., Poletta, G.L., Siroski, P.A., Mudry, M.D., 2012. Baseline values of micronuclei and comet assay in the lizard *Tupinambis merianae* (Teiidae, Squamata). Ecotoxicol. Environ. Saf. 84, 99–103. https://doi.org/10.1016/j.ecoenv.2012.06.023.
Sfakianakis, D.G., Renieri, E., Kentouri, M., Tsatsakis, A.M., 2015. Effect of heavy metals on fish larvae deformities: a review. Environ. Res. 137, 246–255. https://doi.org/10.1016/J.ENVRES.2014.12.014.
Shalan, M.G., 2022. Amelioration of mercuric chloride-induced physiologic and histopathologic alterations in rats using vitamin E and zinc chloride supplement. Heliyon 8 (12), e12036. https://doi.org/10.1016/j.heliyon.2022.e12036.
Shimizu, N., Itoh, N., Utiyama, H., Wahl, G.M., 1998. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. J. Cell Biol. 140 (6), 1307–1320. https://doi.org/10.1083/jcb.140.6.1307.
Silas, E.G., Vijayakumaran, M., Rajagopalan, M., 1984. Yolk utilization in the egg of the olive ridley Lepidochelys olivacea. CMFRI Bull. 35, 22–33. Retrieved from. http://eprints.cmfri.org.in/1066/1/Bulletin_35-3.pdf.
Silva, C., Simões, T., Novais, S.C., Pimparel, I., Granada, L., Soares, A.M.V.M., Barata, C., Lemos, M.F.L., 2017. Fatty acid profile of the sea snail *Gibbula umbilicalis* as a biomarker for coastal metal pollution. Sci. Total Environ. 586, 542–550. https://doi.org/10.1016/j.scitotenv.2017.02.015.
Silva, C.O., Simões, T., Félix, R., Soares, A.M.V.M., Barata, C., Novais, S.C., Lemos, M.F.L., 2021. *Asparagopsis armata* exudate cocktail: the quest for the mechanisms of toxic action of an invasive seaweed on marine invertebrates. Biology 10 (3), 223. https://doi.org/10.3390/biology10030223.
Sinaei, M., Bolouki, M., Ghorbqanzadeh-Zaferani, G., Matin, M.T., Alimoradi, M., Dalir, S., 2018. On a poorly known rookery of green turtles (*Chelonia mydas*) nesting at the Chabahar Beach, Northeastern Gulf of Oman. Russ. J. Mar. Biol. 44 (3), 254–261. https://doi.org/10.1134/S1063074018030094.
Smith, R.C., Reed, V.D., Webb, T.R., 1993. Reversal of copper(II)-induced methemoglobin formation by thiols. J. Inorg. Biochem. 52 (3), 173–182. https://doi.org/10.1016/0162-0134(93)80038-B.
Solís, C., Isaac-Olivéa, K., Martínezb, A., Lavoisierb, E., JA, H.-S., 2007. Trace metals in the sea grass *Thalassia Testudinum* from Mexican Caribbean Coasts. In: Proceedings of the XI International Conference on PIXE and Its Analytical Applications, 1–4 (papers2://publication/uuid/3F409A49-050B-4746-88B6-E69CCC23AE90).
Sönmez, B., 2016. An assessment of egg size in the green turtle (*Chelonia mydas*) on Samandağ Beach, Turkey. Nat. Eng. Sci. 1 (3), 33–41. https://doi.org/10.28978/nesciences.286310.
Sorbera, L.A., Asturiano, J.F., Carrillo, M., Zanuy, S., 2001. Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). Biol. Reprod. 64 (1), 382–389. https://doi.org/10.1095/biolreprod64.1.382.
Souza, N.L.N., Carneiro, M.T.W.D., Pimentel, E.F., Frossard, A., Freire, J.B., Endringer, D. C., Ferreira Júnior, P.D., 2018. Trace elements influence the hatching success and emergence of *Caretta caretta* and *Chelonia mydas*. J. Trace Elem. Med. Biol. 50, 117–122. https://doi.org/10.1016/j.jtemb.2018.06.007.
Speer, R.M., Wise, C.F., Young, J.L., Aboueissa, A.E.M., Martin Bras, M., Barandiaran, M., Bermúdez, E., Márquez-D'Acunti, L., Wise, J.P., 2018. The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in leatherback sea turtle lung cells. Aquat. Toxicol. 198 (March), 149–157. https://doi.org/10.1016/j.aquatox.2018.03.003.
Stacy, N.I., Alleman, A.R., Sayler, K.A., 2011. Diagnostic hematology of reptiles. Clin. Lab. Med. 31 (1), 87–108. https://doi.org/10.1016/j.cll.2010.10.006.
Tahity, T., Islam, M.R.U., Bhuiyan, N.Z., Choudhury, T.R., Yu, J., Noman, M.A., Hosen, M.M., Quraishi, S.B., Paray, B.A., Arai, T., Hossain, M.B., 2022. Heavy metals accumulation in tissues of wild and farmed barramundi from the Northern Bay of Bengal Coast, and its estimated human health risks. Toxics 10 (8). https://doi.org/10.3390/toxics10080410.
Tanabe, L.K., Scott, K., Dasari, V., Berumen, M.L., 2022. An assessment of heavy metals in green sea turtle (*Chelonia mydas*) hatchlings from Saudi Arabia's largest rookery, Ras Baridi. PeerJ 10. https://doi.org/10.7717/peerj.13928.
ter Braak, C.J.F., Šmilauer, P., 2002. CANOCO Reference Manual and CanoDraw for Windows user's Guide: Software for Canonical Community Ordination (Version 4.5). Section on Permutation Methods. Microcomputer Power, Ithaca, New York, p. 10 (https://doi.org/citeulike-article-id:7231853).
Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. In: Reviews in Fisheries Science, vol. 11 (2). Taylor & Francis Group, pp. 107–184. https://doi.org/10.1080/713610925.

Tomlinson, D.L., Wilson, J.G., Harris, C.R., Jeffrey, D.W., 1980. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. Helgoländer Meeresun. 33 (1–4), 566–575. https://doi.org/10.1007/BF02414780/METRICS.

Topping, N.E., Valenzuela, N., 2021. Turtle nest-site choice, anthropogenic challenges, and evolutionary potential for adaptation. Front. Ecol. Evol. 9, 808621 https://doi.org/10.3389/FEVO.2021.808621/BIBTEX.

Valdes, S.A.C., Vieira, L.G., Ferreira, C.H., Mendonça, J.D.S., Ribeiro, P.R.Q., De Abreu Fernandes, E., Santos, A.L.Q., 2015. Effects of exposure to methyl parathion on egg hatchability and eggshell chemical composition in *Podocnemis expansa* (Testudines, Podocnemididae). Zool. Sci. 32 (2), 135–140. https://doi.org/10.2108/zs140164.

van de Merwe, J.P., Hodge, M., Olszowy, H.A., Whittier, J.M., Ibrahim, K., Lee, S.Y., 2009. Chemical contamination of green turtle (*Chelonia mydas*) eggs in Peninsular Malaysia: implications for conservation and public health. Environ. Health Perspect. 117 (9), 1397–1401. https://doi.org/10.1289/ehp.0900813.

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.

Wallace, B.P., Sotherland, P.R., Tomillo, P.S., Bouchard, S.S., Reina, R.D., Spotila, J.R., Paladino, F.V., 2006. Egg components, egg size, and hatchling size in leatherback turtles. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 145 (4), 524–532. https://doi.org/10.1016/j.cbpa.2006.08.040.

Weng, C., Deng, Z.Y., Zhang, N., Zou, Q., Fan, Y.W., Liu, R., Zhen, L.F., Li, J., 2020. Lipid profiles of Chinese soft-shell turtle eggs (*Pelodiscus sinensis*). J. Food Compos. Anal. 94, 103627 https://doi.org/10.1016/j.jfca.2020.103627.

Wiemeyer, S.N., Lamont, T.G., Bunck, C.M., Sindelar, C.R., Gramlich, F.J., Fraser, J.D., Byrd, M.A., 1984. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs-1969-79-and their relationships to shell thinning and reproduction. Arch. Environ. Contam. Toxicol. 13 (5), 529–549. https://doi.org/10.1007/BF01056332.

Yipel, M., Tekeli, İ.O., İşler, C.T., Altuğ, M.E., 2017. Heavy metal distribution in blood, liver and kidneys of Loggerhead (*Caretta caretta*) and Green (*Chelonia mydas*) sea turtles from the Northeast Mediterranean Sea. Mar. Pollut. Bull. 125 (1–2), 487–491. https://doi.org/10.1016/j.marpolbul.2017.08.011.

Zapata, L.M., Bock, B.C., Orozco, L.Y., Palacio, J.A., 2016. Application of the micronucleus test and comet assay in *Trachemys callirostris* erythrocytes as a model for in situ genotoxic monitoring. Ecotoxicol. Environ. Saf. 127, 108–116. https://doi.org/10.1016/j.ecoenv.2016.01.016.