



Lipid and carotenoid quantification in leatherback turtle eggs: insights into reproductive success and conservation

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ABSTRACT: The role of egg nutrients in the embryonic development success of threatened marine reptiles remains relatively unexplored from a conservation perspective. This study assesses lipid and carotenoid profiles in leatherback turtle *Dermochelys coriacea* eggs, which have the lowest hatching success among marine turtles. Correlations between inter-individual nutrient concentrations and reproductive parameters were also investigated. Samples from 47 eggs in 18 clutches from different females were chemically examined at Playa Pacuare Reserve, Costa Rica. Carotenoid concentrations were consistently low compared to other marine turtle species and positively correlated with the proportion of eggs with yolk within each clutch. Larger females deposited eggs with higher proportions of saturated fatty acids, which correlated directly with nest fertility and hatchling size. Our results on the concentrations of lipid nutrients (carotenoids and fatty acids) and their measured correlations with persistent organic pollutants (POPs) and reproductive success indicators reveal that these factors may have a much more significant relevance to reproductive success than previously recognized and deserve further attention from conservation biologists. Integrating egg yolk nutrients into the reproductive scenario of marine turtles opens several avenues for further research. Given the spatial variability of the effects of increasing global pollution and the apparent relationship between nutrients and persistent organic pollutants in egg yolk, studies focusing on improving incubation success should carefully monitor the balance and reproductive effects of these compounds.

KEY WORDS: Lipids · Carotenoids · Pollutants · Global change · Conservation · Reproduction · Sea turtles · Leatherback

1. INTRODUCTION

The leatherback turtle *Dermochelys coriacea* nests in tropical and subtropical beaches worldwide (Stewart & Johnson 2006, Avens et al. 2009) and is globally classified as Vulnerable by the IUCN; however, the Northwest Atlantic subpopulation, which includes the study site, is listed as Least Concern (Wal-

lace et al. 2013). The underlying causes of this global decline, such as in Pacific (97.4%; Spotila et al. 2000, Tapilatu et al. 2013, Griffiths et al. 2024) and Atlantic (Dutton et al. 2005, Vargas et al. 2019) populations, are linked to the impact of conspicuous anthropogenic activities, such as fisheries bycatch and beach mortality, as well as habitat destruction and alteration (Ferraro et al. 2004). Additionally, there may

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be inherent biological limitations to reproduction that are less apparent but require in-depth investigation. For instance, the chemical characteristics, energy content, and nutritional composition of eggs and their effects on embryonic development and hatching physiology may play an important role.

Hatching success rates in leatherback turtle nests are the lowest among marine turtles, with values below 60% (Leslie et al. 1996, Reynolds 2000, Bell et al. 2004, Perrault et al. 2012, Rafferty et al. 2017). Both biotic and abiotic factors on nesting beaches influence hatching success in all sea turtle species (Patino-Martinez et al. 2012, 2014, 2017). However, differences in the diets of sea turtles may influence the nutritional content of eggs and thus differentially affect embryonic development, hatching success, and neonatal phenotype both within and between species.

Leatherback turtles feed in cold, productive waters at northern and southern latitudes (Myers & Hays 2006, Shillinger et al. 2008, Perrault et al. 2014), primarily consuming gelatinous zooplankton such as lion's mane jellyfish *Cyanea capillata* (Davenport & Balazs 1991, James & Herman 2001, Nordstrom et al. 2020).

Egg production and their composition of essential nutrients are directly determined by the diet of the reproductive females (Hipfner et al. 2010), which varies across species and regions (Perrault et al. 2014, Le Gouvello et al. 2017). Notably, egg yolk contains proteins, fatty acids, liposoluble antioxidants (such as vitamins A and E, and carotenoids), and lipids, all of which play crucial roles in embryonic development and hatching success (Watson et al. 2018, Montesana et al. 2021).

Fatty acids are essential for building new cells and embryonic tissues, meeting the energy demands of embryos and neonates, and ultimately contributing to offspring success (Noble et al. 1996, Dunning et al. 2014). These essential lipids are mobilized during reproduction, beginning with their conversion in the liver to initiate vitellogenesis and allowing ovarian follicles to accumulate lipids and fatty acids in the egg yolk (Craven et al. 2008). During development, omega-3 and omega-6 fatty acids contribute to the formation of cell membranes and regulate critical metabolic processes (Ponnampalam et al. 2021). Additionally, these lipids serve as a concentrated energy source, especially vital during the later stages of development when the embryo undergoes rapid growth (Speake et al. 2003).

Carotenoids, as liposoluble antioxidants, protect embryonic tissues from oxidative damage caused by reactive oxygen species (ROS), which are metabolic byproducts generated during development (Surai et

al. 2000). In avian species, higher carotenoid concentrations in the yolk have been linked to improved survival and better-quality offspring (Karadas et al. 2005). Although studies on marine turtles are limited, carotenoids are likely to perform similar functions, particularly in contaminated environments where natural antioxidants are crucial for mitigating the harmful effects of pollutants.

These observations emphasize the importance of conducting species-, population-, and colony-specific evaluations to better understand the combined effects of nutrients and contaminants on reproductive success. As a critical first step and given the limited quantitative data on lipid and carotenoid concentrations in marine turtle eggs, this study provides comprehensive quantitative insights into these nutrients in leatherback turtle eggs. The main objective of this study was to establish a baseline of lipid content, fatty acid profiles, and carotenoid concentrations in leatherback turtle eggs through laboratory analysis of yolk samples. A secondary objective was to determine if inter-clutch variation in these nutrients is associated with differences in persistent organic pollutant (POP) concentrations. This question was motivated by the findings of De Andrés et al. (2016), which reported a negative correlation between POP levels and hatching success in leatherback turtles. Finally, potential relationships between nutrient composition, POP exposure, and reproductive parameters, including hatching success, fertility, viability, and hatchling biometrics, were explored.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 18 clutches from different leatherback turtles *Dermochelys coriacea* were studied between June and August 2008 at Pacuare Reserve, Caribbean Costa Rica (10° 12' 28.19" N, 83° 15' 55.49" W). This nesting area belongs to one of the largest leatherback turtle nesting colonies in the world (Chacón-Chaverri & Eckert 2007, Patino-Martinez et al. 2008, Rivas et al. 2016). To ensure that each clutch belonged to a different female, each study female was identified using at least 1 of these 2 techniques: Monel tags on both rear flippers and PIT (passive integrated transponders) tags on the shoulder of the right front flipper. Nesting females were intercepted at the time of oviposition, during which the following data were recorded: curved carapace length of the female (CCL), curved carapace width of the female (CCW), number of

potentially fertile eggs (PFEs), and number of shelled albumen globes (SAGs; Bell et al. 2004). The number of PFEs and SAGs was determined visually as eggs were deposited into the nest during oviposition. To ensure accurate counting, eggs were manually and temporarily retained during each deposition pulse of the female, following standardized field protocols (Bell et al. 2004). From each clutch, between 1 and 3 randomly selected PFEs were collected ($n = 47$) for subsequent chemical analysis. The collected PFEs were carefully placed in portable insulated containers and transported under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) legal framework to the analytical laboratories at the Doñana Biological Station in Spain. The maximum time between the last sample collection and receipt at the laboratory did not exceed 48 h.

The donor nests of samples were marked and monitored daily on their original beaches until hatchling emergence. For the 11 clutches with emergence (7 were lost due to erosion or flooding), hatching success (proportion of hatched eggs over total PFEs; Eckert & Eckert 1990), fertility rate (proportion of eggs with any degree of embryonic development over total PFEs; Leslie et al. 1996), and viability rate (proportion of PFEs over PFEs + SAGs; De Andrés et al. 2016) were calculated. A random sample of hatchlings from 8 nests (between 4 and 10 per nest) was selected, and biometric measurements of each hatchling were taken, including mass (digital scale with an accuracy of ± 0.1 g), straight carapace length (SCL), and straight carapace width (SCW), measured with a digital caliper (± 0.1 mm accuracy).

Possible correlations between the type of compound and reproductive variables (nest success and neonate biometrics) were explored for all substances analyzed. Detailed information on POPs was obtained from the same eggs analyzed in this study and is documented in a previously published database on the potential effects of POPs on hatching success in leatherback turtles (De Andrés et al. 2016). The POPs analyzed included polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). Specifically, 19 PCB congeners were quantified, comprising 7 ICES PCBs (the 7 congeners selected by the International Council for the Exploration of the Sea [ICES] for environmental monitoring) (28, 52, 101, 138, 153, 170, and 180), 8 mono-ortho PCBs (105, 114, 118, 123, 156, 157, 167, and 189), and 4 non-ortho PCBs (77, 81, 126, and 169). The PBDEs analyzed included both low-brominated congeners (tri- to hexa-BDEs: 28, 47, 66, 85, 99, 100, 153, and 154) and high-brominated congeners (hepta- to deca-BDEs: 183, 184, 191, 196,

197, and 209). These POPs were quantified using gas chromatography-mass spectrometry (GC-MS) techniques, as detailed in the original methodology.

2.2. Chemical analysis

Total lipids were extracted from lyophilized egg samples using chloroform:methanol (2:1, v/v) following the method described by Folch et al. (1957). After solvent evaporation under nitrogen vapor, lipid fractions were dried overnight in a vacuum desiccator and quantified gravimetrically. Total lipids were dissolved in chloroform:methanol (2:1, v/v) with 0.01% butylated hydroxytoluene (BHT) added as an antioxidant and stored at -25°C in nitrogen-purged, sealed vials.

Methyl esters of fatty acids (FAMES) were obtained from total lipid fractions through acid-catalyzed transmethylation (Christie 1982). Nonadecanoic acid (19:0) was used as an internal standard for quantification purposes. After extraction with hexane:diethyl ether (1:1, v/v), FAMES were further purified by thin-layer chromatography (G60 silica gel, Merck) using hexane:diethyl ether:acetic ether (85:15:1.5, v/v/v) as the solvent phase. Purified methyl esters were then dissolved in hexane containing 0.01% BHT and analyzed by gas chromatography (Fisons Instruments 8000 series GC) equipped with a flame ionization detector (FID), a silica capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm film thickness TR-WAX, Teknokroma), and a cold on-column injection system, using helium as the carrier gas. Samples were run under a thermal gradient from 50° to 220°C . Peak identification was conducted by comparison with well-characterized standards (FAME 37, Supelco, Merck KGaA, and Marinol, Fishing Industry Research Institute). All solvents and chemicals were either analytical or HPLC grade. For quantification, a Waters e2695 Alliance chromatograph equipped with a Waters 2998 photodiode array detector was used, operated through Empower2 software (Waters Cromatografía). Calibration levels were established using a range of standard concentrations to ensure accuracy and reproducibility.

Carotenoid extraction from egg yolk was performed following the technique described by Bortolotti et al. (2003). Egg yolk (0.2–0.5 g) was homogenized in 2 ml of a 1:1 (v/v) mixture of 5% NaCl solution and ethanol, followed by the addition of 3 ml of hexane and further homogenization for 3 min. The mixture was centrifuged, the hexane layer was collected, and the extraction was repeated twice. Hexane extracts were combined, evaporated under N_2 ,

the residue was dissolved in 1 ml of methanol:dichloromethane (1:1, v/v), centrifuged, and the supernatant was used for carotenoid determination. The determination of carotenoids was carried out through HPLC, as described by Surai et al. (2000). This process utilized a S3ODS2 Spherisorb reverse-phase column (C18, 5 μ m, 25 cm $\times$ 4.6 mm) supplied by Phase Separation, Clwyd. A gradient elution was employed, with the mobile phase comprising acetonitrile:methanol (85:15) and acetonitrile:dichloromethane:methanol (70:20:10). Detection was performed at an absorbance of 445 nm. Carotenoid peaks were identified by comparing their retention times with those of a variety of carotenoid standards obtained from Sigma, Fluka, Apin, and Hoffman-La Roche, as well as through coelution experiments with these known standards. Quantification was performed using external standard calibration curves generated with purified β -cryptoxanthin, β -carotene, lutein, and zeaxanthin standards previously isolated from natural sources. Standard solutions of these carotenoids were prepared within a concentration range of 0.5–45.0 μ g ml⁻¹. Calibration curves were constructed by plotting the peak area at 445 nm against the pigment concentration to ensure accurate quantification. The sum of all congeners of POPs, including PCBs and PBDEs, was calculated as the total of the mean concentrations of all measured congeners. These values were obtained from the dataset reported in De Andr  s et al. (2016), where POP concentrations in egg yolks were averaged per nest based on all analyzed eggs. Toxicity equivalency factors (TEFs) were not applied, as this study focuses on the overall burden of POPs rather than their relative toxic potency.

2.3. Data analysis

To assess the potential influence of differences among clutches and to determine if the egg sampling was representative of the population, similarity analyses (ANOSIM) using the Bray-Curtis index were conducted in PRIMER software (v 6.1.6). Each compound was treated as a separate variable, and each egg was considered as an individual sample for intra-clutch variability analyses. Additionally, to better visualize differences among and within clutches, a non-metric multidimensional scaling (NMDS) plot was generated using PRIMER software (v 6.1.6; van de Merwe et al. 2010, De Andr  s et al. 2016).

Potential outliers were examined using the Grubbs test, comparing eggs within the same clutch, with eggs from other clutches, and with values reported in

the literature. To establish baseline data for the different compounds, the average concentrations and proportions across all clutches were calculated.

Descriptive statistics, including mean, standard deviation, median, and range, were calculated for all compound groups. Proportional data, such as reproductive rates (viability, fertility, and hatching success) and fatty acid proportions, were arcsine-transformed following established guidelines to stabilize variance and meet assumptions of normality (Mosteller & Youtz 1961).

The normality of all variables was assessed using the Shapiro-Wilk test (Shapiro & Wilk 1965). Most variables followed a normal distribution ($p > 0.05$), including all reproductive parameters and hatchling morphometrics, except for fertility rate, which was analyzed using non-parametric Spearman rank correlations.

Regarding POP variables, normality tests indicated that hepta- to deca-BDEs and all PCB groups did not follow a normal distribution. For these variables, a log₁₀ transformation was applied to improve data distribution for statistical analyses. Normality was reassessed using the Shapiro-Wilk test, and parametric or non-parametric correlations were applied accordingly. This transformation was particularly relevant given that PCB concentrations were an order of magnitude higher than PBDEs, and both POP groups exhibited high variability, ensuring a more balanced representation in the statistical analyses. Additionally, correlations between female size (measured as CCL and CCW) and reproductive parameters (e.g. clutch size, hatching success) as well as hatchling morphometrics (e.g. straight carapace width) were evaluated to control for potential effects of maternal size on these outcomes.

All statistical analyses, including descriptive summaries, normality testing, outlier identification, and correlation assessments, were performed using STATISTICA software (v 8.0) with a significance level of $\alpha = 0.05$.

3. RESULTS

3.1. Nutrient composition and reproductive parameters

A summary of reproductive parameters, egg yolk composition, and hatchling morphometrics for the 18 study nests is provided in Table 1. The mean clutch size (excluding SAGs) was (mean $\pm$ SD) 79 $\pm$ 11 eggs, with viability and fertility rates of 0.69 $\pm$ 0.09 and

Table 1. Reproductive parameters, yolk composition (per clutch), and hatchling morphometrics in leatherback turtles. Clutch size: number of eggs with yolk; viability rate: clutch size/(clutch size + SAGs); fertility rate: fertile eggs/clutch size). Total lipid content expressed in mg g^{-1} dry mass; carotenoid concentrations are in ng g^{-1} wet mass. Persistent organic pollutants (POPs) are measured in ng g^{-1} lipid. Hatchling morphometrics include straight carapace length (SCL) and straight carapace width (SCW). (–): missing hatchling emergence data

ID female/clutch	Clutch size	Viability rate	Fertility rate	Lipids (mg g^{-1})	Carotenoids (ng g^{-1})	POPs (ng g^{-1})	Mass (g)	SCL (mm)	SCW (mm)	Mass/ SCL (g mm^{-1})
1	(–)	(–)	(–)	50.3	861.4	105.5	(–)	(–)	(–)	(–)
2	73	0.63	0.96	55.1	165.3	100.4	49.5	(–)	(–)	(–)
3	99	0.66	(–)	44.9	810.0	133.1	(–)	(–)	(–)	(–)
4	76	0.66	0.88	56.7	819.4	100.5	(–)	(–)	(–)	(–)
5	78	0.67	0.78	58.0	694.1	62.0	(–)	(–)	(–)	(–)
6	85	0.79	(–)	57.2	1379.5	82.4	(–)	(–)	(–)	(–)
7	59	0.69	0.76	49.7	(–)	88.4	45.6	57.5	39.2	0.79
8	69	0.63	0.96	59.2	1088.4	65.6	46.1	58.0	38.9	0.80
9	96	0.66	0.78	50.1	973.8	146.4	49.2	60.3	40.5	0.82
10	77	0.65	(–)	44.8	343.5	108.4	(–)	(–)	(–)	(–)
11	79	0.90	0.78	53.4	529.6	32.2	41.6	56.7	37.5	0.73
12	74	0.61	0.95	47.3	207.5	135.8	50.5	60.6	34.5	0.83
13	82	0.67	0.95	62.0	0.0	72.2	49.0	54.2	36.3	0.90
14	80	0.73	(–)	36.2	1107.2	223.7	(–)	(–)	(–)	(–)
15	56	0.52	(–)	54.3	277.0	441.5	(–)	(–)	(–)	(–)
16	80	0.84	0.90	52.2	1063.9	236.6	43.5	56.4	38.0	0.77
17	73	0.60	0.84	46.5	45.0	27.4	(–)	(–)	(–)	(–)
18	100	0.75	(–)	54.2	614.9	31.1	(–)	(–)	(–)	(–)
Mean	79	0.69	0.87	51.8	645.9	121.8	46.88	54.84	40.67	0.81
SD	± 11	± 0.09	± 0.08	± 6.3	± 416.7	± 98.5	± 3.19	± 6.60	± 8.79	± 0.05

0.87 ± 0.08 , respectively. The mean ($\pm$ SD) lipid content was $51.8 \pm 6.3 \text{ mg g}^{-1}$ dry weight and that of carotenoids was $645.9 \pm 416.7 \text{ ng g}^{-1}$ wet weight. The sum of all congeners of POPs per clutch ranged from 27.4 to 441.5 ng g^{-1} lipid, with an average across all clutches of $121.9 \pm 98.5 \text{ ng g}^{-1}$ lipid. The sum of all congeners of POPs per clutch was calculated as the total of the mean concentrations of all measured PCB and PBDE congeners, ranging from 27.4 to 441.5 ng g^{-1} lipid, with mean ($\pm$ SD) across all clutches of $113.9 \pm 96.5 \text{ ng g}^{-1}$ lipid. These values were derived from the dataset reported in De Andrés et al. (2016), where POP concentrations in egg yolks were averaged per nest based on all analyzed eggs. TEFs were not applied, as this study focuses on the overall burden of POPs rather than their relative toxic potency.

3.2. Fatty acids

Monounsaturated fatty acids accounted for nearly half of the total fatty acids (mean $\pm$ SD: $45.1 \pm 1.8\%$, range: 41.4–49.2%), followed by saturated fatty acids ($33.8 \pm 1.2\%$, 30.9–36.1%). Polyunsaturated fatty

acids (PUFAs) represented $11.8 \pm 2.1\%$, with n-3 levels slightly lower than n-6 ($5.1 \pm 1.1\%$, 3.4–9.5% vs. $5.6 \pm 0.8\%$, 4.3–8.3%, respectively). The remaining percentage of other PUFAs was $1.1 \pm 0.2\%$ (range 0.7–1.5%). The total concentration of FAMES was 321.6 mg g^{-1} dry weight of total lipid content in the egg yolk.

The fatty acids with the highest mean concentrations were oleic acid (31.6%), followed by palmitic acid (15.8%) and myristic acid (10.7%; Table 2). Palmitoleic acid, cis-vaccenic acid, and stearic acid showed similar proportions around 6%, while arachidonic acid accounted for 3.7%. Docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3) were present at mean concentrations of 1.2 and 2.9%, respectively (Table 2).

Variations in n-3 PUFA concentrations were positively and significantly correlated with nest fertility rates (Fig. 1). Additionally, a highly significant correlation was observed between saturated fatty acid concentrations and SCW of hatchlings ($R = 0.92$, $p = 0.003$; Fig. 2). Saturated fatty acid concentrations were higher in eggs from larger females (CCL; $R = 0.51$, $p < 0.05$).

Table 2. Fatty acid composition (%) and carotenoid concentrations (ng g⁻¹ wet mass) in leatherback turtle egg yolks (n = 47 eggs). n (C > 0) represents the number of eggs with carotenoid concentrations above the detection limit

Compound/name	Designation	Mean	SD	Min.	Max.
Fatty acids					
Myristic	<14	5.3	0.7	3.5	6.8
Pentadecanoic	14:0	10.7	0.8	8.5	11.9
Palmitic	15:0	0.7	0.1	0.5	0.8
Palmitoleic	16:0	15.8	1.0	13.5	17.0
Hexadecatrienoic	16:1n-7	6.1	0.5	4.9	7.6
Heptadecanoic	16:3	1.0	0.2	0.5	1.3
Stearic	17:0	1.1	0.2	0.5	1.4
Oleic	18:0	5.5	0.6	4.4	6.7
cis-Vaccenic	18:1n-9	31.6	1.5	28.2	34.9
Linoleic	18:2n-6	5.9	0.4	5.1	6.8
α-linoleic	18:3n-3	1.5	0.3	1.1	2.0
Gondoic	20:1n-9	0.3	0.1	0.2	0.5
Arachidonic	20:4n-6	1.1	0.3	0.7	1.9
Eicosapentaenoic	20:5n-3	3.7	0.7	2.6	6.2
Docosapentaenoic	22:5n-3	0.5	0.2	0.2	1.5
Docosahexaenoic	22:6n-3	1.2	0.5	0.5	3.5
		2.9	0.4	2.2	3.8
Carotenoids	n (C > 0)				
Zeaxanthin	38	481.1	342.2	0.0	1220.3
Lutein	8	32.9	79.7	0.0	358.6
Unidentified 1	34	156.6	152.2	0.0	652.2
Unidentified others	11	26.1	49.8	0.0	192.6
Total carotenoids	41	696.7	488.5	0.0	1811.7

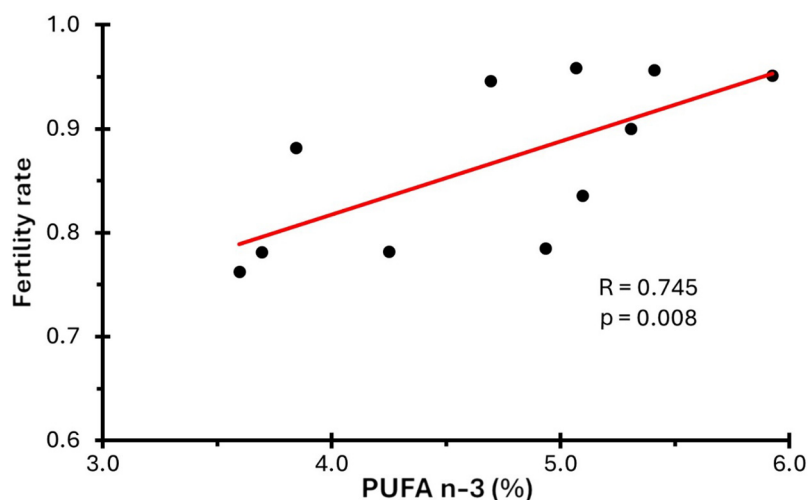


Fig. 1. Significant positive correlation between the proportion of n-3 polyunsaturated fatty acids (PUFA n-3) in leatherback turtle egg yolk and nest fertility rate (n = 11 clutches; Spearman's rank correlation: R = 0.745, p = 0.008). This relationship suggests a potential role of PUFA n-3 in enhancing reproductive success in leatherback turtles

3.3. Carotenoids

The average concentrations of carotenoids (ng g⁻¹ wet weight) showed a wide range of variation (0–1379) with a mean (±SD) value of 645.9 ± 416.7. Zea-

xanthin had the highest concentrations (mean = 481.1, maximum = 1220.3; Table 2), followed by 'unidentified 1' (mean = 156.6, maximum = 652.2; Table 2). Both pigments exhibited a high number of cases above the detection limit (38 and 34, respectively). However, lutein had only 8 cases above the detection limit with a mean concentration of 32.9 (Table 2). Zeaxanthin concentrations were positively correlated with viability (R = 0.52, p < 0.05; Fig. 3).

3.4. Intra and inter-clutch variability

Lipid and carotenoid profiles did not differ significantly among clutches (ANOSIM-R values = 0.47 for lipids, 0.31 for carotenoids, p > 0.05). In contrast, a wide range of intra-clutch variation was observed in the concentration profiles of lipids and carotenoids. The intra- and inter-clutch clustering patterns observed among sampled turtles are graphically represented in the 2D-NMDS plots in Fig. S1 in the Supplement at www.int-res.com/articles/suppl/n057p009_suppl.pdf.

3.5. Environmental and reproductive parameters

Inter-clutch variation in total POP concentrations was positively and significantly related to quantitative differences in PUFA concentrations (R = 0.47, p = 0.04). n-6 PUFAs were positively correlated with POP concentrations normalized by lipids (R = 0.62, p = 0.007). Conversely, there was no significant correlation between POP concentrations and carotenoid concentrations or hatchling morphometry. There was a direct relationship between CCL of females and CCW of

hatchlings (R = 0.82, p < 0.05). Viability rate was inversely correlated with hatchling mass (R = -0.896, p = 0.003). Hatching success was negatively correlated with the mass-to-carapace length ratio of hatchlings (R = -0.94, p = 0.002).

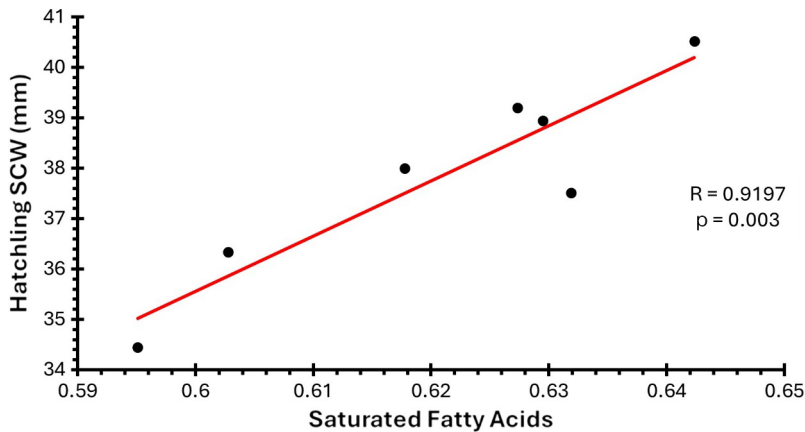


Fig. 2. Significant positive correlation between the arcsine-transformed proportion of saturated fatty acids in leatherback turtle egg yolk ($n = 7$ clutches) and the straight carapace width (SCW) of hatchlings ($R = 0.9197$, $p = 0.003$). This suggests that higher saturated fatty acid content in the egg yolk is associated with larger hatchling size

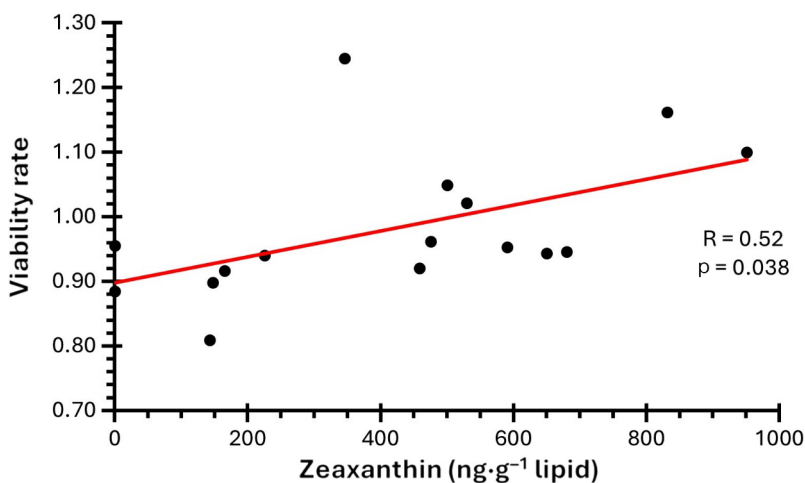


Fig. 3. Significant positive correlation between zeaxanthin concentration in leatherback turtle egg yolk (mean per clutch) and nest viability rate ($R = 0.52$, $p = 0.038$). This suggests that higher zeaxanthin levels may contribute to improved nest viability

4. DISCUSSION

This study provides the first quantification of lipid content, fatty acid profiles, and carotenoid concentrations in leatherback turtle *Dermochelys coriacea* eggs. These compounds, considered crucial in reptile reproductive physiology (Thompson & Speake 2002, Price 2017), enable both intra- and interspecific comparisons across broader geographical contexts. Our findings, which reveal significant correlations between these compounds, POPs, and reproductive success indicators, underscore the unexpected relevance of these interactions and their implications for conservation biology, as discussed below.

Carotenoids, as lipid-soluble antioxidants, play a critical role in protecting embryonic tissues from peroxidative damage (Surai & Speake 1998). Increasing ROS associated with global warming and thermal stress threaten embryonic viability, heightening the importance of carotenoids as key mitigators of oxidative stress (Parisi & Guerriero 2019). However, this area of study remains largely unexplored among sea turtles (Weber 2010, De Andrés et al. 2016), despite substantial evidence from birds (Speake et al. 1999b, Nys 2000, Newbrey et al. 2008, Ruuskanen et al. 2011, Sinanoglou et al. 2011) and some data from reptiles (Speake et al. 2001, Dierenfeld et al. 2002, Weiss et al. 2011, Reinke et al. 2018). In the only study assessing carotenoid concentrations in sea turtle hatchlings, Weber (2010) found that larger hatchlings originated from eggs with higher carotenoid concentrations, which significantly decreased across successive clutches, potentially due to physiological limitations in maternal reserves. Furthermore, yolk carotenoid levels were not associated with hatchling resistance to oxidative stress or with adaptive responses to developmental environments, challenging the assumption that carotenoid deposition serves as an adaptive maternal effect. Our study provides the first published records of carotenoid concentrations in leatherback turtle eggs, revealing levels 7 times lower than those in green turtles

(leatherback = [mean $\pm$ SD] $0.7 \pm 0.5 \mu\text{g g}^{-1}$ of fresh yolk, green = $5.14 \pm 0.4 \mu\text{g g}^{-1}$ of fresh yolk; Weber 2010), and up to 17 times lower than those in freshwater turtles *Testudo hermanni* and *Emydura macquarii* (Thompson et al. 1999, Speake et al. 2001). These low levels are consistent with the jellyfish-based diet of leatherback turtles, which is characterized by minimal pigmentation (Davenport & Balazs 1991, James & Herman 2001, Takaichi 2011, Viera et al. 2018). Zeaxanthin, the most abundant pigment in our samples, was significantly associated with viability rates among clutches, suggesting a potential role in vitellogenesis. Further research is needed to explore whether carotenoids like zeaxanthin influence

vitellogenin production, yolk formation, and overall embryo development. Additionally, factors like oviductal conditions, maternal age, migratory effort and environmental variability may contribute to these patterns, underscoring their complexity (Wallace et al. 2004, Caut et al. 2006, Patino-Martinez et al. 2010).

Lipids are the primary energy source for embryonic development and initial mobility in sea turtles (Thompson & Speake 2002) and account for 44–70% of the yolk content (Craven et al. 2008, Castro-González & Pérez-Gil 2011). In leatherback turtles, the relative abundance of long-chain PUFAs like docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3) highlights their nutritional importance, particularly for neural and retinal development (Speake et al. 2003, Hulbert 2005). These fatty acids may confer adaptive advantages by meeting the high energetic demands of embryos in the ecological niche of leatherback turtles (Surai et al. 2000). Our findings show higher proportions of saturated fatty acids in eggs from larger females, likely reflecting greater energy reserves due to increased food intake and storage capacity (Kwan 1994). These saturated fats were positively correlated with larger hatchlings, potentially enhancing survival and contributing to reproductive success (Gyuris 2000). Some of the fatty acids analyzed in our study support this thesis; for example, n-6 PUFAs were positively correlated with neonate length, or n-3 PUFAs with nest fertility, as observed in other species (e.g. *Penaeus chinensis*; Xu et al. 1994). Wide differences in fatty acid proportions have been found among sea and freshwater turtle species studied so far. For instance, Thompson et al. (1999) found arachidonic acid (20:4 n-6) levels 3 times lower in the freshwater turtle *Emydura macquarii* than those found in this study for leatherback turtles at 1.2 versus 3.7%, respectively. Arachidonic acid is found in the cell membranes of embryonic organs such as the brain, liver, and heart, and play a regulatory role (Speake & Thompson 1999, Speake et al. 1999a). Therefore, understanding the roles of the nutritional components of the egg in fertility, embryonic growth success, biometrics, and offspring survival may be key in conservation biology.

Although studies demonstrating correlations between lipid nutrients (carotenoids and fatty acids) and POP mobilization remain scarce, there is extensive evidence of the detrimental effects of POPs on sea turtle reproductive success (Keller et al. 2004, Alava et al. 2006, Guirlet et al. 2010, van de Merwe et al. 2010, Stewart et al. 2011, De Andrés et al. 2016, Du Preez et al. 2018, Souza et al. 2018). This study found that POPs interact with key metabolic compounds,

such as PUFAs, potentially due to their accumulation in lipid-rich tissues and subsequent transfer to egg yolks during vitellogenesis. This interaction highlights the dual role of POPs: on the one hand, they bioaccumulate in lipid-rich tissues and are transferred to eggs during vitellogenesis. On the other hand, they potentially interfere with nutrient metabolism and oxidative stress pathways (Alava et al. 2006). The significant correlation observed between n-6 PUFA levels and POP concentrations highlights the propensity of POPs to accumulate in fatty tissues and their role in exacerbating oxidative damage. Such damage has been linked to developmental disruptions and decreased reproductive success in marine species (Costantini 2010). However, while these correlations were statistically significant, their strength was moderate, suggesting that additional factors may influence these interactions. Thus, our findings should be interpreted as an initial step toward understanding the broader metabolic effects of POP exposure in *D. coriacea*, rather than as definitive conclusions. Further research is needed to investigate additional biochemical and physiological variables that may mediate these processes, as well as to assess the specific contributions of individual POP congeners rather than their total sum. Furthermore, the absence of a significant relationship between carotenoid concentrations and POP levels supports the hypothesis that oxidative and metabolic impacts of these contaminants vary depending on the nutrient type, with lipophilic nutrients being more directly affected by contaminant bioaccumulation (Craven et al. 2008). The persistence and biomagnification of POPs in marine food webs, along with their presence in nesting environments, contribute to long-term exposure risks for sea turtles (Arienzo 2023). For leatherback turtles, these risks may be exacerbated by their extensive migratory patterns and trophic levels, which increase their exposure to lipophilic contaminants. To address these challenges, biomarker studies should monitor oxidative damage and reproductive impacts in turtle populations exposed to POPs (Arienzo 2023). The balance between nutrient availability and contaminant stressors influences embryonic development and the adaptability of sea turtle populations facing increasing environmental pressures. Prospectively, the relationship between POPs and PUFAs (particularly n-6) could amplify the detrimental effects of POPs on reproduction, further highlighting the need for targeted conservation efforts and more extensive studies on the biochemical mechanisms underpinning these interactions. These include, for example, elucidating the balance be-

tween the positive effects of n-3 and n-6 PUFAs and the negative effects of POPs and investigating the combined influence of compounds of different nature and with different properties on embryonic development and ultimately on individual reproductive success and their influence on population conservation. Furthermore, studying the effects of environmental changes in feeding areas and the nutritional characteristics of egg components at both intra- and inter-population levels that could differentially affect their adaptive potential to global environmental changes; seeking predictable global associations on reproductive success of different species and their diet, and unravelling the underlying biological mechanisms of such associations. However, the entry of egg nutrients into the reproductive scenario also poses some difficulties in relation to the traditionally implicit assumption underlying studies on sea turtle reproductive success, i.e. that the characteristics of the environment on nesting beaches mostly or exclusively affect their reproductive success (Patino-Martinez et al. 2022).

Due to the spatial variability of the effects associated with increasing global contamination and the connection found here between essential nutrients and POPs present in egg yolk, it is crucial that studies focused on improving incubation success pay careful attention to the interaction between these compounds. This complex relationship suggests the need to understand how these elements affect egg yolk quality for successful incubation and production of healthy hatchlings (Morão et al. 2024). This comprehensive approach is fundamental to addressing the challenges related to the conservation of threatened migratory oviparous species in a context of global environmental change.

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