Oxidative stress is a potential cost of synchronous nesting in olive ridley sea turtles

B. Gabriela Arango^{1,2*}, Martha Harfush-Meléndez³, David C. Ensminger¹, Elpidio Marcelino López-Reyes^{3,4}, José Alejandro Marmolejo-Valencia⁵, Horacio Merchant-Larios⁵, Daniel E. Crocker², José Pablo Vázquez-Medina¹

1) Department of Integrative Biology, University of California, Berkeley, 2) Department of Biology, Sonoma State University, 3) Centro Mexicano de la Tortuga, Mazunte, Oaxaca, México, 4) Campamento Tortuguero Palmarito, Puerto Escondido, Oaxaca, México, and 5) Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México

*Correspondence:

B. Gabriela Arango
Vázquez-Medina Lab
Department of Integrative Biology
University of California, Berkeley
3040 Valley Life Sciences Bldg #3140,
Berkeley CA 94720-3140

Abstract

Olive ridley sea turtles, *Lepidochelys olivacea*, exhibit a polymorphic reproductive behaviour nesting in solitary or in mass aggregations termed “arribadas”, where thousands of turtles nest at once. Arribadas may provide fitness benefits including mate finding during nearshore aggregations and predator satiation at the time of hatching, but the potential costs of arribada nesting remain understudied. To explore the potential trade-offs of the fitness benefits associated with arribada nesting, we collected blood from olive ridley turtles nesting in arribada and solitary. We measured reproductive and metabolic hormones (progesterone, estradiol, testosterone, thyroxine, and triiodothyronine), triglycerides (TG), non-esterified fatty acids (NEFA) and markers of oxidative damage (4-hydroxynonenal, malondialdehyde, protein carbonyls, and nitrotyrosine). Arribada nesters were bigger and had higher levels of progesterone than solitary nesters. Similarly, thyroid hormones were higher in individuals nesting in arribada than in solitary nesters, while TG and NEFA were positively correlated in arribada but not in solitary nesters. Nesting in arribada was associated with increased lipid peroxidation and protein carbonyls compared to solitary nesting. These results suggest that nesting in arribada is potentially more energetically expensive than nesting solitarily, and that oxidative stress may be a trade-off of the fitness benefits associated with arribada nesting.

Keywords: reproduction, life history theory, protein oxidation, metabolic cost

Introduction

Sea turtles of the genus *Lepidochelys*, which includes Kemp's (*Lepidochelys kempi*) and olive ridley turtles (*Lepidochelys olivacea*), exhibit an unusual reproductive polymorphic behaviour, nesting both in solitary and in massive aggregations termed "arribadas" [1]. During arribada nesting, thousands of turtles nest synchronously over a 2-7 day interval [1–3]. Notably, the same individual can nest interchangeably in arribadas or solitary, but the factors that determine whether an individual joins the arribada or nests in solitary remain unknown [2,4].

Olive ridley turtles nesting in arribadas can extend their inter-nesting intervals up to 63 days by inducing hypoxia-driven pre-ovipositional embryonic arrest so they can synchronize to nest [5,6]. Environmental cues that might trigger arribadas include the moon phase, weather patterns, and rainfall [2,7]. Although the exact benefits of arribada nesting are unknown, this behaviour provides a social context that promotes multiple mating and paternity, increasing genetic exchange by three-fold compared to solitary nesting [2,8–10]. Furthermore, nesting in arribada increases early hatchling dispersal by overwhelming or satiating predators [11]; however, arribada nesting does not necessarily promote hatchling success as increased organic matter from egg saturation and high nesting density in arribada beaches result in lower hatchling success compared to solitary nesting [3,12,13]. In contrast, solitary nesters produce bigger hatchlings, which have higher male-to-female ratio than hatchlings produced by arribada nesters [1,6]. Thus, while both nesting modes are likely important for the population, it is unknown if the potential fitness benefits associated with arribada nesting come with a physiological cost.

A central principle of life history theory is that reproductive effort negatively affects survival; however, there is conflicting evidence about the proximal costs of reproduction [14]. One of the leading hypotheses suggests that oxidative stress is a potential trade-off for reproductive investment [15–17], but other studies do not support this idea [18,19]. Work in wild vertebrates suggests that diversification of reproductive strategies is associated with sexual differences in oxidative stress [20–22]. In male and female free-ranging macaques [23], male northern elephant seals [24] and female North American red squirrels [25], reproduction causes oxidative stress. Similarly, nesting decreases the activity of the antioxidant superoxide dismutase in female loggerhead sea turtles [26].

To the best of our knowledge, the potential trade-offs of arribada nesting have not been studied. Therefore, we compared the circulating concentrations of reproductive and metabolic hormones, and markers of oxidative stress in olive ridley turtles nesting solitary and in arribada. We found that arribada nesters are heavier and have higher metabolic activity and oxidative damage than solitary nesters. Thus, our results suggest that oxidative stress is a potential cost of arribada nesting in olive ridley sea turtles.

Methods

Animals and sample collection

Animal handling protocols were approved by Sonoma State University's IACUC. Samples were collected under permit SGPA/DGVS/12915/16 and imported to the US under permits CITES MX88143 and CITES 19US85728C/9. Nesting olive ridley turtles were sampled at the marine protected area of *La Escobilla*, Oaxaca, Mexico (15° 47' N; 96° 44' W) during arribada (n = 13). Solitary nesting turtles were sampled at *Campamento Tortuguero Palmarito*, Puerto Escondido, Oaxaca, México (15° 53' 26.3" N; 97° 07' 52.2" W), and *La Escobilla* during solitary nesting (n = 10). Samples were collected after the animals had dug their nest, during the 'trance-nesting period' [27]. None of the animals were disturbed from nesting, nor returned to the ocean without laying their eggs. Mass was estimated using a regression from published olive ridley morphometric data (n= 59, mass = -47.44 + 1.13 x straight carapace length (SCL), $r^2 = 0.70$, $p < 0.001$; [28]). Animals sampled during solitary nesting were weighed using a hand-held scale (± 0.1 kg) to assess the validity of the mass-estimation method. The equation used to estimate mass predicted the

weight of solitary nesters with a mean error of 4%. Blood was collected from the cervical vein into chilled heparin Vacutainer tubes [27,29]. Plasma was prepared by centrifugation onsite, frozen, and subsequently stored at -20°C until laboratory analysis.

Biochemical Assays

Hormones: Progesterone (P4), estradiol (E2), thyroxine (T4), triiodothyronine (T3) and testosterone were measured in sea turtle serum using ELISA or RIA kits (progesterone: MP Biomedical ELISA catalog number 07BC1113, estradiol: MP Biomedical ELISA catalog number 07BC1111, T3: MP Biomedical RIA catalog number 06B-254215, T4: MP Biomedical RIA catalog number 06B-254011, testosterone: Enzo ELISA catalog number ADI-900-065). Assay platforms were validated for use in olive ridley turtles. Serially diluted pooled samples (1:2 to 1:16) exhibited parallelism to the standard curve after log-logit transformation. Mean recovery of hormone added to serum pools was $101.5 \pm 3.5\%$, $98.3 \pm 6.5\%$, $99.4 \pm 4.7\%$, and for $101.3 \pm 5.1\%$ ($r^2 > 0.98$) for P4, E2, T3 and T4, respectively. The testosterone kit was previously validated for sea turtle blood [30] and was further validated for our samples using parallelism. Assays were conducted following manufacturer's instructions.

Oxidative damage: Two markers of lipid peroxidation (4-hydroxynonenal: 4-HNE, malondialdehyde: MDA), a marker of protein oxidation (protein carbonyls), and a marker of protein nitration (3-nitrotyrosine) were measured in sea turtle plasma using ELISA kits (Cell Biolabs catalog numbers: STA-838, STA-832, STA-310, STA-305). Assays were conducted following the manufacturer's instructions with minor modifications as previously described [31].

Plasma lipids and total protein: Plasma triglycerides (TG) and non-esterified fatty acids (NEFA) were measured using colorimetric kits (TG: Cayman Chemical catalog number 10010303, NEFA: Wako Chemicals, HR Series NEFA-HR (2) catalog number: 999-34691). Total protein content was measured using a bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific catalog number: A53227). Oxidative damage values were normalized to total plasma protein levels.

All samples were analyzed in duplicate in a single assay. The average intra-assay coefficient of variation was <6%.

Statistical analyses

Statistical analyses were conducted using JMP Pro 15 (SAS Institute, USA). Equality of variances was assessed using Levene's test. Progesterone, TG and NEFA data were log10 transformed to meet model assumptions. Arribada and solitary nesting groups were compared using two-sample t-tests. A nominal logistic regression was used to analyze 4-HNE data since the response variable was binary. Correlation analyses between hormones, mass, and metabolites including those measured in samples from the same animals in a previous study [32], were conducted by calculating Pearson or Spearman coefficients (for corticosterone non-parametric data). Data are expressed as mean $\pm$ SD unless otherwise indicated. Statistical significance was considered at $p \leq 0.05$. Figures were generated using R packages ggplot2, plotly, scales and ggthemes.

Results

Arribada nesters have higher progesterone levels than solitary nesters

We measured circulating progesterone, estradiol, and testosterone to assess reproductive hormone profiles in solitary and arribada nesters. Progesterone was higher in arribada than in solitary nesters (3.58 ± 1.71 vs 2.10 ± 1.56 ng/mL, $t = 2.612$, $p = 0.02$; **Figure 1A**). Estradiol did not differ between nesting conditions (solitary 4.47 ± 2.79 vs arribada 5.7 ± 2.24 pg/mL, $t = -1.17$, $p = 0.25$; **Figure 1B**). Similarly,

testosterone was not different between solitary and arribada nesters (70.43 ± 42.88 vs 75.08 ± 35.99 pg/mL, $t=-0.29$, $p=0.78$; **Figure 1C**). These results show that females nesting in arribadas have higher progesterone but not estradiol or testosterone levels than solitary nesters.

Metabolic activity is higher in arribada than in solitary nesters

We quantified plasma T4 and T3 to evaluate whether arribada nesting induces a potential change in metabolic activity compared to solitary nesting. Both T3 and T4 were higher in individuals nesting in arribada than in solitary nesters (T3: 48 ± 19.54 vs 29.3 ± 11.86 ng/dL, $t = 2.66$, $p = 0.015$; T4: 1.45 ± 0.27 vs 1.12 ± 0.13 ug/dL, $t = 3.62$, $p = 0.002$; **Figure 2A, 2B**), suggesting increased thyroid function in arribada nesters. While neither TG nor NEFA levels were different between nesting modes (TG: solitary 14.46 ± 9.52 vs arribada 20.47 ± 10.03 mg/mL, $t=1.94$, $p=0.07$; NEFA: solitary 0.93 ± 0.22 vs arribada 1.08 ± 0.55 mM, $t=0.445$, $p=0.66$), arribada nesters were heavier than solitary nesters (29.20 ± 5.20 vs 24.76 ± 4.37 kg, $t=2.17$, $p=0.042$). We then conducted correlation analyses between hormones and metabolites including those (corticosterone, glucose and lactate) measured in samples from the same individuals in a previous study [32]. We found a positive correlation between corticosterone and glucose ($\rho=0.87$, $p<0.0001$; **Figure 3A**), corticosterone and lactate ($\rho=0.73$, $p=0.005$; **Figure 3B**), glucose and lactate ($r=0.85$, $p=0.0003$; **Figure 3C**), and TG and NEFA ($r=0.82$, $p=0.0005$; **Figure 3D**) in arribada but not in solitary nesters. These results suggest that arribada nesters have higher metabolic activity than solitary nesters.

Oxidative damage is a potential cost of arribada nesting

We then evaluated whether oxidative damage represents a proximate cost for nesting in arribada by measuring four circulating markers of oxidative damage. Plasma levels of the lipid peroxidation products 4-HNE (arribada 100% positive vs solitary 25% positive for 4-HNE, $\chi^2(2)=10.01$, $p=0.0016$), and MDA (0.0025 ± 0.0013 vs 0.0011 ± 0.00034 pmol/ μ g of protein, $t=3.77$, $p=0.0019$), along with protein carbonyls (0.00246 ± 0.0013 vs 0.00103 ± 0.00032 nmol/ μ g of protein, $t=3.64$, $p=0.0027$) were higher in arribada than in solitary nesters (**Figures 4A, 4B, 4C**). In contrast, protein nitration (3-Nitrotyrosine) did not vary between nesting modes (solitary 0.039 ± 0.026 vs arribada 0.058 ± 0.031 nM/mg, $t=-1.29$, $p=0.22$; **Figure 4D**). These results suggest that oxidative damage is a potential cost of synchronous nesting in olive ridley sea turtles.

Discussion

Every year thousands of olive ridley sea turtles synchronize in “arribadas” to nest over a 2-7 day period. The fitness benefits associated with arribada nesting include mate finding during nearshore aggregations [2], predator satiation at the time of hatchling [11], and multiple paternity and genetic exchange [8]. Whether arribada nesting carries a physiological cost remains unknown. Here, we found that arribada nesters have higher progesterone levels, metabolic activity and oxidative stress than solitary nesters. Therefore, our results suggest that nesting in arribada may be energetically more expensive than nesting in solitary and that oxidative damage is a trade-off for the potential fitness benefits associated with arribada nesting in olive ridley sea turtles.

Potential endocrine drivers of arribada nesting

Environmental cues that trigger arribada events include the moon phase, temperature, humidity and salinity [2,7], but the exact combination of factors remains unknown and it is likely population-specific [7,33]. Similarly, endogenous adjustments that allow individuals to synchronize to join an arribada are poorly understood. Long-term egg retention is uniquely observed in the genus *Lepidochelys* spp. [5], where average inter-nesting intervals last about 28 days while inter-nesting in other species lasts 9-15 days [5]. Moreover, eggs from olive ridley turtles nesting in arribada can tolerate hypoxia-driven pre-ovipositional embryonic arrest for longer periods compared to eggs from solitary nesters (15 vs 4 days)

[6]. Increased concentrations of gonadosteroids might also influence whether an individual joins the arribada. We found higher progesterone but not estradiol or testosterone levels in arribada than in solitary nesters. Previous work, however, shows that neither progesterone nor estradiol influence the holding of arribada eggs inside the reproductive tract [34], and that inter-nesting differences among *Lepidochelys* spp. and other sea turtles are not related to differences in ovulatory cycles, but rather to egg retention capacity [5,35]. A progesterone surge during ovulation induces a rapid albumen release into the oviduct, which activates previously stored sperm [36]. Therefore, rather than exerting endocrine control over synchronicity, it is possible that high progesterone levels are related to increased mating opportunities in arribada compared to solitary nesters. Of note, progesterone levels do not differ with nesting mode in Kemp's ridley turtles [37]. Thus, there is no conclusive evidence about potential endocrine drivers of arribada nesting in olive ridley sea turtles.

Is arribada nesting more energetically expensive than solitary nesting?

In a previous study conducted in the same individuals we showed that arribada nesters have higher circulating corticosterone and glucose levels than solitary nesters [32]. Here, we found that arribada nesters are bigger and have higher thyroid hormone levels than solitary nesters. Moreover, we found correlations between corticosterone and glucose, corticosterone and lactate, glucose and lactate, and TG and NEFA levels in arribada but not in solitary nesters. Thus, our combined results suggest that arribada nesters have larger energy reserves and higher metabolic activity than solitary nesters. Our results are consistent with those reported in animals nesting in the Rushikulya Rookery of Orissa, India, where arribada nesters are also larger than solitary nesters [38]. As discussed above, the same individual can nest interchangeably in arribadas or in solitary, but the factors that determine whether an individual joins the arribada or nests in solitary remain unknown [2,4]. Our results suggest that arribadas are energetically more expensive than solitary nesting and that bigger animals with larger energetic reserves join the arribadas. As capital breeders [39–41], sea turtles feed and build their energy reserves prior to migrating, mating and nesting. Hence, if arribada nesting is more energetically costly than solitary nesting, individuals lacking appropriate energy reserves might choose solitary nesting over joining the arribadas despite losing the potential fitness benefits associated with arribada nesting. Solitary nesting requires shorter inter-nesting intervals. Thus, solitary nesting likely results in less time spent away from the feeding grounds [34], potentially allowing solitary nesters to build their energy reserves and join the arribadas later. Therefore, potential differences in resource availability and allocation might ultimately affect whether an individual joins the arribada. According to life history theory, reproduction is a costly life history trait, and our results suggest that nesting in arribada is more energetically expensive than nesting in solitary, though it carries potential fitness benefits such as increased genetic exchange [8].

Oxidative damage is a potential trade-off of the fitness benefits associated with arribada nesting

The cost of reproduction represents one of the most fundamental life history trade-offs [16], but there is inconclusive evidence about whether oxidative stress is a proximal cost of reproductive investment [14]. Some results support this idea [15–17], while others refute it [18,19]. Increased reproductive effort can promote susceptibility to oxidative stress derived from either exhaustion of antioxidant defenses or dysregulated oxidant generation due to increased metabolic activity, but work with laboratory versus wild animals often yields conflicting results [42]. Moreover, differences in reproductive strategies within wild vertebrates likely result in differential susceptibility to oxidative stress [20–22]. As capital breeders, sea turtles depend on stored energy to fuel reproductive expenditure [43,44]. Whether capital breeders have higher susceptibility to oxidative stress than income breeders remains unknown. In free ranging macaques [23] and North American red squirrels [25] reproduction increases oxidative stress. In contrast, reproduction does not affect lipid peroxidation (measured as thiobarbituric acid reactive substances) in Children's pythons [45], but in aspic vipers [46] and northern elephant seals, reproduction increases oxidative stress despite concurrent upregulation of antioxidant defenses [24]. Here we found higher lipid

peroxidation and protein oxidation levels in arribada compared to solitary nesters. We also found that arribada nesting is potentially more energetically costly than solitary nesting. These results suggest that higher energy expenditure in olive ridley turtles nesting in arribadas is associated with increased oxidative stress. In northern elephant seals, breeding increases lipid peroxidation and oxidative DNA damage in males but not in females [24]. Elephant seals are polygynous, sexually dimorphic capital breeders, with adult males being up to eight times heavier than females [47,48]. Males compete for position in a dominance hierarchy used to control access to females [47–50]. Thus, energy expenditure associated with breeding is higher in male than in female elephant seals [51]. Hence, in capital breeders with unique reproductive behaviours that result in increased energy expenditure, oxidative stress might be a trade-off for potential fitness benefits associated with such behaviours.

In conclusion, we found that arribada nesting in olive ridley sea turtles is associated with increased levels of circulating oxidative damage compared to solitary nesting. Moreover, our results suggest that arribada nesters have higher metabolic activity than solitary nesters. Thus, oxidative stress may be a trade-off for a reproductive mode that carries increased fitness benefits in a capital breeder. Whether these trade-offs are present in other capital breeders remains unknown and undoubtedly warrants further investigation.

Acknowledgements

We thank Centro Mexicano de la Tortuga and Campamento Tortuguero Palmarito personnel, especially Ernesto Albavera-Padilla for his assistance with sampling logistics and useful discussions, and Allison Raymundo, Carmelo Ambrosio, Alberto Jarquín and Antonio Santiago for their assistance during fieldwork. We thank Cooperativa la Tortuga Feliz, Heradio-Santillán and Sóstenes Rodríguez-Reyes from La Escobilla Campamento Tortuguero, and Dr. Javier Beltran-Robledo for their assistance with fieldwork and sample processing.

Funding

B.G.A. was supported by the UC Berkeley Chancellor's Fellowship. José Alejandro Marmolejo-Valencia and Horacio Merchant-Larios were funded by UNAM (PAPIIT-IN201218). Research funded by UC Berkeley startup funds.

Author's contributions

Conceptualization: B.G.A and J.P.V.-M. Data curation: B.G.A., D.C.E., and D.E.C. Formal analysis: B.G.A. Investigation: B.G.A., D.C.E., M.H-M., and E.M.L-R. Methodology: B.G.A., D.C.E., H.M-L., E.M.L-R., and D.E.C. Project Administration: J.A.M-V, H.M-L. Resources: M.H-M., E.M.L-R., H.M-L., D.E.C, and J.P.V.M. Supervision: J.P.V.M. Writing – original draft: B.G.A. and J.P.V.M. Writing – review and editing: J.P.V.M. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Figure legends

Figure 1. Progesterone levels are higher in arribada than in solitary nesters. Reproductive hormone levels in solitary and arribada nesters. **A)** Progesterone ($p = 0.02$), **B)** estradiol ($p = 0.25$), and **C)** testosterone concentrations ($p = 0.78$).

Figure 2. Thyroid hormones are higher in arribada than in solitary nesters. **A)** Thyroxine ($p = 0.002$) and **B)** triiodothyronine ($p = 0.015$) levels in solitary and arribada nesters.

Figure 3. Correlations between hormones and metabolites in arribada and solitary nesters. **A)** corticosterone and glucose (arribada: $p=0.87$, $p<0.0001$), **B)** corticosterone and lactate (arribada: $p=0.73$,

p=0.005), **C**) glucose and lactate (arribada: $r=0.85$, $p=0.0003$), **D**) TG and NEFA (arribada: $r=0.61$, $p=0.0021$).

Figure 4. Oxidative damage is higher in arribada than in solitary nesters.

Lipid peroxidation measured as **A**) 4-HNE-protein adducts (binary test, percent positive arribada, 100% vs solitary, 25%, $p = 0.0009$, $\chi^2(2)=10.01$, $p=0.0016$), and **B**) MDA-protein adducts ($p = 0.0019$); **C**) protein carbonyls ($p = 0.0027$) and **D**) 3-Nitrotyrosine ($p = 0.22$).

REFERENCES

1. Dornfeld TC, Robinson NJ, Tomillo PS, Paladino F V. 2015 Ecology of solitary nesting olive ridley sea turtles at Playa Grande, Costa Rica. *Mar. Biol.* **162**, 123–139. (doi:10.1007/s00227-014-2583-7)
2. Bernardo J, Plotkin PT. 2007 An evolutionary perspective on the arribada phenomenon and reproductive behavioral polymorphism of olive ridley sea turtles (*Lepidochelys olivacea*). In *Biology and Conservation of Ridley Sea Turtles* (ed PT Plotkin), pp. 59–87. Baltimore: The Johns Hopkins University Press.
3. Ocana M, Harfush-Melendez M, Heppell S. 2012 Mass nesting of olive ridley sea turtles *Lepidochelys olivacea* at La Escobilla, Mexico: linking nest density and rates of destruction. *Endanger. Species Res.* **16**, 45–54. (doi:10.3354/esr00388)
4. Hirth HF. 1980 Some aspects of the nesting behavior and reproductive biology of sea turtles. *Integr. Comp. Biol.* **20**, 507–523. (doi:10.1093/icb/20.3.507)
5. Plotkin PT, Rostal DC, Byles RA, Owens DW. 1997 Reproductive and Developmental Synchrony in Female *Lepidochelys olivacea*. *J. Herpetol.* **31**, 17–22.
6. Williamson SA, Evans RG, Robinson NJ, Reina RD. 2019 Synchronised nesting aggregations are associated with enhanced capacity for extended embryonic arrest in olive ridley sea turtles. *Sci. Rep.* **9**, 9783. (doi:10.1038/s41598-019-46162-3)
7. Bézy VS, Putman NF, Umbanhowar JA, Orrego CM, Fonseca LG, Quirós-Pereira WM, Valverde RA, Lohmann KJ. 2020 Mass-nesting events in olive ridley sea turtles: environmental predictors of timing and size. *Anim. Behav.* **163**, 85–94. (doi:10.1016/j.anbehav.2020.03.002)
8. Jensen MP, Abreu-Grobois FA, Frydenberg J, Loeschcke V. 2006 Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. *Mol. Ecol.* **15**, 2567–2575. (doi:10.1111/j.1365-294X.2006.02951.x)
9. Plotkin PT, Byles RA, Rostal DC, Owens DW. 1995 Independent versus socially facilitated oceanic migrations of the olive ridley, *Lepidochelys olivacea*. *Mar. Biol.* **122**, 137–143. (doi:10.1007/BF00349287)
10. Plotkin PT, Owens DW, Byles RA, Patterson R. 1996 Departure of Male Olive Ridley Turtles (*Lepidochelys olivacea*) from a Nearshore Breeding Ground. *Herpetologica* **52**, 1–7.
11. Eckrich CE, Owen DW. 1995 Solitary versus Arribada Nesting in the Olive Ridley Sea Turtles (*Lepidochelys Olivacea*): A Test of the Predator-Satiation Hypothesis. *Herpetologica* **51**, 349–354.
12. Bézy VS, Valverde RA, Plante CJ. 2014 Olive Ridley Sea Turtle Hatching Success as a Function of Microbial Abundance and the Microenvironment of In Situ Nest Sand at Ostional, Costa Rica. *J. Mar. Biol.* **2014**, 1–10. (doi:10.1371/journal.pone.0118579)
13. Honarvar S, O'Connor MP, Spotila JR. 2008 Density-dependent effects on hatching success of the olive ridley turtle, *Lepidochelys olivacea*. *Oecologia* **157**, 221–230. (doi:10.1007/s00442-008-1065-3)
14. Metcalfe NB, Monaghan P. 2013 Does reproduction cause oxidative stress? An open question. *Trends Ecol. Evol.* **28**, 347–350. (doi:10.1016/J.TREE.2013.01.015)
15. Alonso-Alvarez C, Bertrand S, Devevey G, Prost J, Faivre B, Sorci G. 2004 Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecol. Lett.* **7**, 363–368. (doi:10.1111/j.1461-0248.2004.00594.x)
16. Dowling DK, Simmons LW. 2009 Reactive oxygen species as universal constraints in life-history evolution. *Proc. R. Soc. B Biol. Sci.* **276**, 1737–1745. (doi:10.1098/rspb.2008.1791)
17. Monaghan P, Metcalfe NB, Torres R. 2009 Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecol. Lett.* **12**, 75–92. (doi:10.1111/j.1461-0248.2008.01258.x)
18. Garratt M, Vasilaki A, Stockley P, McArdle F, Jackson M, Hurst JL. 2011 Is oxidative stress a physiological cost of reproduction? An experimental test in house mice. *Proc. R. Soc. B Biol. Sci.* **278**, 1098–1106. (doi:10.1098/rspb.2010.1818)
19. Ołdakowski Ł, Piotrowska Z, Chrzęścik KM, Sadowska ET, Koteja P, Taylor JRE. 2012 Is reproduction costly? No increase of oxidative damage in breeding bank voles. *J. Exp. Biol.* **215**, 1799–1805. (doi:10.1242/jeb.068452)
20. Birnie-Gauvin K, Costantini D, Cooke SJ, Willmore WG. 2017 A comparative and evolutionary

- approach to oxidative stress in fish: A review. *Fish Fish.* **18**, 928–942. (doi:10.1111/faf.12215)
21. Costantini D. 2018 Meta-analysis reveals that reproductive strategies are associated with sexual differences in oxidative balance across vertebrates. *Curr. Zool.* **64**, 1–11. (doi:10.1093/cz/zox002)
22. Godoy RS, Lanés LEK, Castro BD, Weber V, Wingen N, Pires MM, Oliveira GT, Maltchik L. 2020 Oxidative stress resistance in a short-lived Neotropical annual killifish. *Biogerontology* **21**, 217–229. (doi:10.1007/s10522-019-09855-w)
23. Georgiev A V., Thompson ME, Mandalaywala TM, Maestriperi D. 2015 Oxidative stress as an indicator of the costs of reproduction among free-ranging rhesus macaques. *J. Exp. Biol.* **218**, 1981–1985. (doi:10.1242/jeb.121947)
24. Sharick JT, Vazquez-Medina JP, Ortiz RM, Crocker DE. 2015 Oxidative stress is a potential cost of breeding in male and female northern elephant seals. *Funct. Ecol.* **29**, 367–376. (doi:10.1111/1365-2435.12330)
25. Fletcher QE, Selman C, Boutin S, McAdam AG, Woods SB, Seo AY, Leeuwenburgh C, Speakman JR, Humphries MM. 2013 Oxidative damage increases with reproductive energy expenditure and is reduced by food-supplementation. *Evolution (N. Y.)*. **67**, 1527–1536. (doi:10.1111/evo.12014)
26. Perrault JR, Stacy NI. 2018 Note on the unique physiologic state of loggerhead sea turtles (*Caretta caretta*) during nesting season as evidenced by a suite of health variables. *Mar. Biol.* **165**, 1–6. (doi:10.1007/s00227-018-3331-1)
27. Dutton PH. 1996 Methods for collection and preservation of samples for sea turtle genetic studies. In *Proceedings of the International Symposium* (eds BW Bowen, WN Witzell), pp. 17–24. Miami, Florida. (doi:10.1051/parasite/2014024)
28. Espinoza-Romo BA, Sainz-Hernández JC, Ley-Quinónez CP, Hart CE, Leal-Moreno R, Aguirre AA, Zavala-Norzagaray AA. 2018 Blood biochemistry of olive ridley (*Lepidochelys olivacea*) sea turtles foraging in northern Sinaloa, Mexico. *PLoS One* **13**, 1–12. (doi:10.1371/journal.pone.0199825)
29. Mettee N. 2014 Sample Collection Techniques. See <http://www.seaturtleguardian.org/sample-collection-techniques>.
30. Allen CD *et al.* 2015 First assessment of the sex ratio for an east pacific green sea turtle foraging aggregation: Validation and application of a testosterone ELISA. *PLoS One* **10**, 1–25. (doi:10.1371/journal.pone.0138861)
31. Vazquez-Medina JP, Zenteno-Savin T, Tift MS, Forman HJ, Crocker DE, Ortiz RM. 2011 Apnea stimulates the adaptive response to oxidative stress in elephant seal pups. *J. Exp. Biol.* **214**, 4193–4200. (doi:10.1242/jeb.063644)
32. Arango BG, Harfush-Meléndez M, Marmolejo-Valencia JA, Merchant-Larios H, Crocker DE. 2020 Blood oxygen stores of olive ridley sea turtles, *Lepidochelys olivacea* are highly variable among individuals during arribada nesting. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* , 1–10. (doi:10.1007/s00360-020-01321-1)
33. Pritchard PCH. 2007 Arribadas I Have Known. In *Biology and Conservation of Ridley Sea Turtles* (ed PT Plotkin), pp. 7–22. Baltimore: Johns Hopkins University Press.
34. Owens DW, Morris YA. 1985 The Comparative Endocrinology of Sea Turtles. *Copeia* **3**, 723–735.
35. Licht P, Owens DW, Clifton K, Penaflores C. 1982 Changes in LH and progesterone associated with the nesting cycle and ovulation in the olive ridley sea turtle, *Lepidochelys olivacea*. *Gen. Comp. Endocrinol.* **48**, 247–253. (doi:10.1016/0016-6480(82)90023-5)
36. Owens DW. 1980 The comparative reproductive physiology of sea turtles. *Integr. Comp. Biol.* **20**, 549–563. (doi:10.1093/icb/20.3.549)
37. Rostal DC, Grumbles JS, Byles RA, Marquez-M R, Owens DW. 1997 Nesting Physiology of Kemp's Ridley Sea Turtles, *Lepidochelys kempi*, at Rancho Nuevo, Tamaulipas, Mexico, with Observations on Population Estimates. *Chelonian Conserv. Biol.* **2**, 538–547.
38. Tripathy B. 2008 An assessment of solitary and arribada nesting of olive ridley sea turtles (*Lepidochelys olivacea*) at the Rushikulya rookery of Orissa, India. *Asiat. Herpetol. Res.* **11**, 136–142.
39. Goldberg DW *et al.* 2013 Ghrelin and leptin modulate the feeding behaviour of the hawksbill turtle *Eretmochelys imbricata* during nesting season. *Conserv. Physiol.* **1**, 1–13. (doi:10.1093/conphys/cot016)
40. Hamann M, Limpus CJ, Whittier JM. 2002 Patterns of lipid storage and mobilisation in the female green sea turtle (*Chelonia mydas*). *J. Comp. Physiol. B* **172**, 485–493. (doi:10.1007/s00360-002-

- 0271-2)
41. Plot V, Jenkins T, Robin J-P, Fossette S, Georges J-Y. 2013 Leatherback Turtles Are Capital Breeders: Morphometric and Physiological Evidence from Longitudinal Monitoring. *Physiol. Biochem. Zool.* **86**, 385–397. (doi:10.1086/671127)
42. Ensminger DC, Salvador-pascual A, Arango BG, Allen KN, Vazquez-Medina JP. 2021 Fasting ameliorates oxidative stress : A review of physiological strategies across life history events in wild vertebrates. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **256**. (doi:10.1016/j.cbpa.2021.110929)
43. Bonnet X, Bradshaw D, Shine R. 1998 Capital versus Income Breeding : An Ectothermic Perspective. *Oikos* **83**, 333–342.
44. Stephens PA, Boyd IL, McNamara JM, Houston AI. 2009 Capital breeding and income breeding: their meaning, measurement, and worth. *Ecology* **90**, 2057–2067.
45. Stahlschmidt ZR, Lourdais O, Lorient S, Butler MW, Davis JR, Salin K, Voituron Y, Denardo DF. 2013 Morphological and physiological changes during reproduction and their relationships to reproductive performance in a capital breeder. *Physiol. Biochem. Zool.* **86**, 398–409. (doi:10.1086/670918)
46. Stier A, Dupoué A, Picard D, Angelier F, Brischoux F, Lourdais O. 2017 Oxidative stress in a capital breeder (*Vipera aspis*) facing pregnancy and water constraints. *J. Exp. Biol.* **220**, 1792–1796. (doi:10.1242/jeb.156752)
47. Deutsch CJ, Haley MP, Le Boeuf BJ. 1990 Reproductive effort of male northern elephant seals: estimates from mass loss. *Can. J. Zool.* **68**, 2580–2593. (doi:10.1139/z90-360)
48. Deutsch CJ, Crocker DE, Costa DP, Le Boeuf BJ. 1994 Sex- and age-related variation in reproductive effort of northern elephant seals. In *Elephant seals: population ecology, behavior, and physiology* (eds BJ Le Boeuf, RM Laws), pp. 169–210. Berkeley: University of California Press. (doi:10.1525/9780520328150-012)
49. Haley MP, Deutsch CJ, Le Boeuf BJ. 1994 Size, dominance and copulatory success in male northern elephant seals, *Mirounga angustirostris*. *Anim. Behav.* **48**, 1249–1260. (doi:10.1006/ANBE.1994.1361)
50. Le Boeuf BJ. 1974 Male-male Competition and Reproductive Success in Elephant Seals. *Am. Zool.* **14**, 163–176. (doi:10.1093/icb/14.1.163)
51. Crocker DE, Houser DS, Webb PM. 2012 Impact of Body Reserves on Energy Expenditure, Water Flux, and Mating Success in Breeding Male Northern Elephant Seals. *Physiol. Biochem. Zool.* **85**, 11–20. (doi:10.1086/663634)

434 **Fig. 1.**

a

b

c

435

Fig. 2.

a

b

Fig. 3.

Fig. 4.