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ORIGINAL PAPER

Blood oxygen stores of olive ridley sea turtles, *Lepidochelys olivacea* are highly variable among individuals during arribada nesting

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Abstract

Sea turtles dive with a full lung of air and these O_2 stores are supplemented by O_2 stored in blood and muscle. Olive ridley sea turtles exhibit polymorphic nesting behavior, mass nesting behavior called arribada, where thousands of turtles will nest at once, and solitary nesting behavior. The potential physiological differences between the individuals using these strategies are not well understood. We measured blood volume and associated variables, including blood hemoglobin content and hematocrit, to estimate total blood O_2 stores. There were no significant differences in mean values between nesting strategies, but arribada nesting individuals were more variable than those performing solitary nesting. Mass-specific plasma volume was relatively invariant among individuals but mass specific blood volume and blood oxygen stores varied widely, twofold and threefold, respectively. Blood O_2 stores represented 32% of total body O_2 stores. Under typical mean diving conditions of 26 °C and high levels of activity, blood stores confer ~ 14 min to aerobic dive times and are likely critical for the long duration, deep diving exhibited by the species. Individual differences in blood O_2 stores strongly impact estimated aerobic dive limits and may constrain the ability of individuals to respond to changes on ocean climate.

Keywords Blood oxygen stores · Olive ridley · cADL · Arribada nesting · Solitary nesting

Abbreviations

ADL	Aerobic dive limit
BV	Blood volume
cADL	Calculated aerobic dive limit
Hct	Hematocrit
Mb	Myoglobin
MCHC	Mean corpuscular hemoglobin concentration
PV	Plasma volume

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Introduction

Air-breathing diving animals display a variety of anatomical, physiological, and behavioral adaptations that allow them to increase their time underwater used for foraging, migrating, mating and predator avoidance. These animals maximize time at depth through the use of aerobic metabolism and enhance dive durations through increased body O_2 stores (Kooyman 1989). Total body O_2 stores are dependent on several variables, including diving lung volume, blood volume, hemoglobin (Hb) concentration, muscle mass, and myoglobin (Mb) concentration (Ponganis 2011). While the O_2 stores and the physiology of breath-hold diving have been investigated extensively in endotherms, less is known about individual and inter-specific variation in diving O_2 stores in marine reptiles, including sea turtles (Berkson 1966; Wells and Baldwin 1994; McMahon et al. 2007).

As ectotherms, body temperature and rates of O_2 consumption are also influenced by water temperature in the Cheloniidae sea turtles (Hochscheid et al. 2004). Understanding the factors that limit sea turtle breath-hold capacity is important to predicting their ability to respond to potential changes in water temperature and prey distributions that

result from climate change (Yang et al. 2019) and the potential for mortality due to incidental by-catch in fishing nets, the main anthropogenic threat to sea turtles (Polovina et al. 2003, 2004; Wallace et al. 2011).

Another key difference among marine divers is whether or not they make use of their lung O_2 stores. The deepest divers, including many marine mammals, dive below the depth of lung collapse and prioritize tissue O_2 stores. Some deep divers, like phocid seals, avoid respiratory gas exchange at depth by diving after exhalation (Ponganis 2011). Other marine divers, such as sea turtles and penguins, dive following inhalation (Wood et al. 1984), and lung O_2 stores are an important component of diving metabolism (McDonald and Ponganis 2013). In sea turtles, the lung serves as the major O_2 store during diving and limited measurements of tissue O_2 stores have suggested similar values to non-diving vertebrates (Lutz and Bentley 1985; Lutcavage et al. 1990, 1992). For this reason, the literature in sea turtles has de-emphasized measurements of blood O_2 stores with most published values from small samples, captive animals or juveniles.

Several recent studies suggest the importance of including blood O_2 stores in the consideration of breath-hold ability in sea turtles (Wells and Baldwin 1994; Hochscheid et al. 2007; McMahon et al. 2007; Chambault et al. 2016). Biologging data have revealed deep, long-duration dives in some species that may exceed the capacity of lung O_2 stores to support aerobic metabolism (McMahon et al. 2007; Chambault et al. 2016). Lung collapse may occur in deep-diving turtles due to their compliant respiratory system on the deepest dives evident in biologging studies (Tenney et al. 1974; Lutcavage et al. 1989) and pulmonary shunts during diving may limit access to lung O_2 stores (Garcia Párraga et al. 2018). Together, these findings suggest that contribution of tissue O_2 stores to aerobic limits may be critical for some dives. Further, recent studies on the sympathetic autonomic control of pulmonary shunts to limit decompression injuries in sea turtles may limit access to lung O_2 stores in some contexts and the lung stores are accessed using intermittent perfusion (Garcia Párraga et al. 2018).

The aerobic dive limit (ADL) is an estimation of physiological and energetic constraints on the dive durations of air-breathing diving animals. ADL is the maximum amount of time an animal can spend underwater before the product of anaerobic respiration, lactate, rises beyond resting levels in their blood (Costa et al. 2001). However, establishing a direct and precise ADL value through measurements of blood lactate is difficult to achieve. Therefore, ADL is calculated as cADL, which is the sum of the total usable amount of O_2 stored in the body (O_2 from blood, muscles and lungs), divided by the diving metabolic rate (Ponganis et al. 2011). Values for cADL based on measurements of oxygen stores have only been reported in leatherbacks (*Dermochelys coriacea*) (Lutcavage et al. 1992; Southwood et al. 1999; Wallace et al. 2005; Bradshaw et al. 2007) and loggerheads (*Caretta caretta*) (Hochscheid et al. 2005).

Despite being the smallest sea turtle, olive ridleys (*Lepidochelys olivacea),* exhibit long duration, deep dives when foraging pelagically. Mean dive duration for a large sample of adult females was 46 min (Chambault et al. 2016). Dives as long as 200 min (3.3 h) and as deep as 420 m have been reported (Polovina et al. 2003; McMahon et al. 2007; Da Silva et al. 2011; Chambault et al. 2016). To our knowledge, data for the calculation of total blood O_2 stores in the sea turtle, olive ridley have not been reported in the literature, except for one small sample ($n = 2$) of blood volume measurements (Thorson 1968).

Olive ridley turtles exhibit two types of nesting behavior, solitary nesting and arribada nesting (Bernardo and Plotkin 2007). Solitary nesting occurs throughout the year and females are widely spaced, returning at varying inter-nesting intervals (Dornfeld et al. 2015). During arribada nesting, thousands of females synchronize to nest together, during the third-quarter moon for a 2–7 day arribada period (Bernardo and Plotkin 2007). Studies have suggested that arribada nesting may confer anti-predator benefits from predator–satiation (Eckrich and Owen 1995) and may confer fitness benefits through multiple matings and paternity (Williamson et al. 2019). However, offspring mortality may be increased at very high densities (Ocana et al. 2012) and density-dependent effects on environmental variables may have played a role in the decline of arribadas in some locations (Honarvar et al. 2008). In general, hatchling success is much lower at arribada beaches than in solitary nesters (< 35 vs $> 75\%$), suggesting solitary nesting may play important roles in maintaining populations (Bézy et al. 2014; Dornfeld et al. 2015). Additionally, arribada beaches might be important in the production of females, given their higher nesting temperatures (Valverde et al. 2010), while solitary beaches might be important in the production of males for the opposite reason (Dornfeld et al. 2015). Arribada and solitary nesters have distinct inter-nesting intervals, the time between two successful nesting events, (3 vs. 4 weeks) (Williamson et al. 2019). However, the physiological and behavioral differences between individuals exhibiting the two strategies are not well understood.

Our primary objectives were to (1) measure plasma and blood volume and estimate blood O_2 stores in large sample of free- ranging adult female olive ridley sea turtles during nesting; (2) compare blood O_2 stores and associated variables between the arribada and solitary nesting strategies; (3) estimate cADL for the sampled olive ridley sea turtles at a range of water temperatures and activity

levels and compare these estimates to published diving behavior.

Methods

Field procedures

All animal handling procedures were approved by the Sonoma State University IACUC and performed under collecting permit SGPA/DGVS/12915/16. Samples were exported for laboratory analysis to Sonoma State University under exporting/importing permits CITES MX88143 and CITES 19US85728C/9. Nesting olive ridley females were sampled at the marine protected area of La Escobilla, Oaxaca, Mexico (15° 47′ N; 96° 44′ W; Fig. 1) during arribada in November 2017 ($n = 13$), and solitary nesting turtles were sampled both at Campamento Tortuguero Palmarito, Puerto Escondido, Oaxaca, México (15° 53′ 26.3″ N; 97° 07′ 52.2″ W), and at La Escobilla during solitary nesting in February 2017 ($n = 10$). Handling and sampling procedures were performed on nesting turtles, after digging their nest, since this is when females enter a 'trance-nesting period' (Dutton 1996) and we were less likely to disrupt nesting. None of the females were disturbed from their nesting, nor returned to the sea without laying their eggs.

Straight carapace length and width were measured for all females. Females sampled during solitary nesting were weighed using a hand-held scale (±0.1 kg). Because of equipment failure, it was not possible to weigh the females sampled during arribada nesting. For these females, mass was estimated using a regression from published data on olive ridley morphometrics (Espinoza-Romo et al. 2018; $n = 59$, mass $= -47.44 + 1.13 \times$ straight carapace length (SCL), $r^2 = 0.70$, $p < 0.001$.). This equation predicted the mass of the turtles that were weighed with a mean error of 4%.

All blood samples were collected from the cervical vein (Owens and Ruiz 1980). An initial blood sample was taken into a 5 ml heparin Vacutainer tube. This sample was used for all hematological and plasma measurements. For plasma volume measurements, females were injected with Evan's blue dye (~1 mg/kg) and 3 sequential samples were taken into heparin tubes at ~7 min intervals after dye injection. Blood samples were kept on ice for 20 min until centrifuged

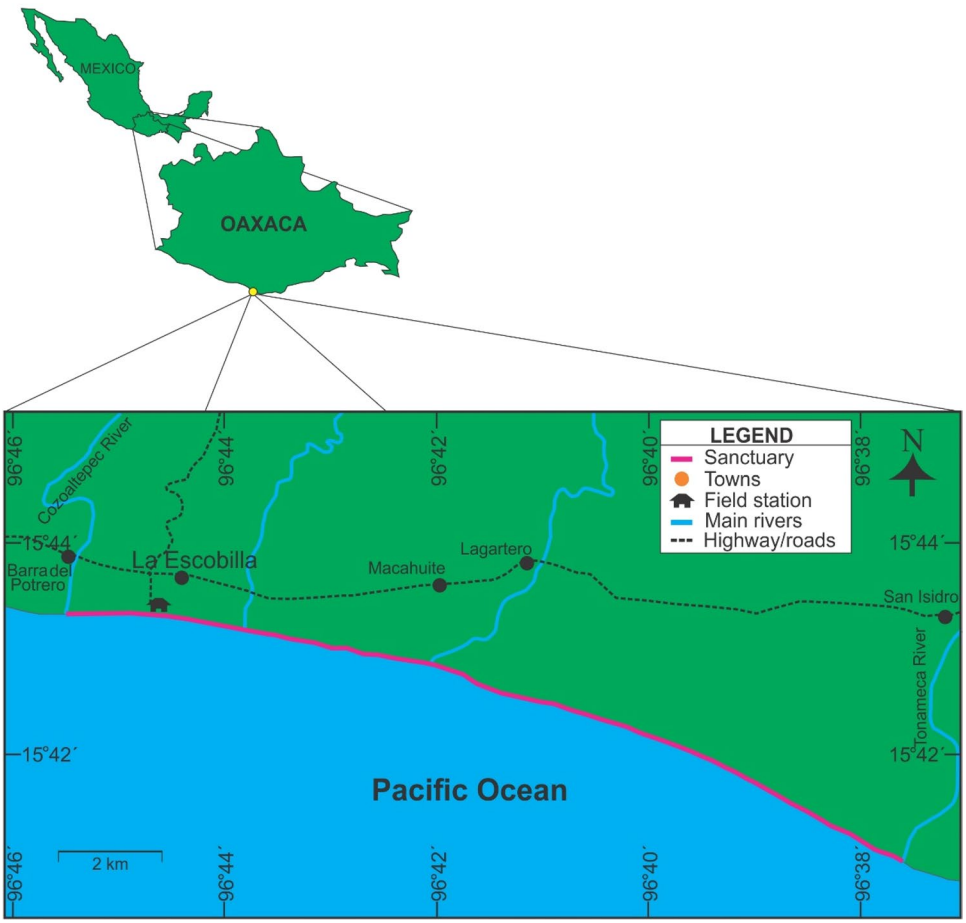

Fig. 1 Marine Protected Area La Escobilla. Field site located in the Pacific Ocean in Oaxaca, Mexico. Figure by E. Albavera-Padilla

at the field site. The initial sample was used to measure hematocrit in triplicate using a clinical hematocrit centrifuge. All plasma samples were frozen after centrifugation at −20 °C until transported on dry ice to the laboratory at Sonoma State University. After transport, samples were stored at −80 °C until analysis. All samples were analyzed within 1 month of return.

Laboratory procedures

Blood O_2 stores

Hemoglobin (Hb) concentration was measured using the cyanmethemoglobin method (Sigma D5941, St. Louis, MO). Mean corpuscular hemoglobin concentration (MCHC) was calculated as Hb/Hct and expressed as 100 Hb Hct^{-1}. Plasma Volume (PV) was estimated using the dilution curve of the injected Evan's Blue dye (El-Sayed et al. 1995). The syringes used to inject Evan's blue were calibrated gravimetrically to calculate the exact injection volume. Absorbance of Evans blue dye in the plasma was determined at 624 and 740 nm. Using the pre-injection samples, values at 740 nm were used to calculate the blank optical density at 624 nm and correct for hemolysis and precipitate in the post-injection samples (Foldager and Blomqvist 1991). Values from serially collected samples were log-transformed, fit to a regression line, and the *y*-intercept was used to determine the instantaneous dilution volume by comparing to a serial dilution of the injectate (El-Sayed et al. 1995). Blood volume (BV) was calculated as PV (1 − Hct/100), where PV is absolute plasma volume. Total available blood O_2 stores were calculated assuming one-third of blood was arterial and two-thirds of blood was venous (Lutz and Bentley 1985), 95 and 80% saturation, respectively (Lutcavage et al. 1990, 1992), that arterial stores were depleted to 20% saturation and a Hb O_2 carrying capacity of 1.34 ml O_2 g^{-1}.

Total O_2 stores and cADL

Muscle and lung O_2 stores were estimated from mass and combined with usable blood O_2 stores to estimate total O_2 stores. No data for muscle myoglobin (Mb) exist for olive ridleys, so we used a published value from their closes relative, *Lepidochelys kempii*. Muscle O_2 stores were estimated assuming 22% of mass is muscle (Lutz and Bentley 1985) and a Mb concentration of 3.1 mg g^{-1} muscle tissue (Stabenau and Heming 1994). Lung O_2 stores were estimated using an allometric equation for lung volume (Hochscheid et al. 2007),

$$lungO_2(ml) = 0.174(113.6 \times Mass^{0.923})$$

and assuming the inspired air was 17.4% O_2 (Berkson 1966). O_2 consumption rate was estimated using the equation developed for captive loggerhead turtles that incorporates water temperatures and activity levels (Kinoshita et al. 2018). Finally, cADL was calculated as the total usable body O_2 stores divided by the O_2 consumption rate. We calculated cADL for each individual turtle at 100% activity and a mean water temperature of 26 °C, the average temperature recorded for free-ranging olive ridleys (Chambault et al. 2016). We calculated and plotted cADL for total O_2 stores and for the contribution of blood O_2 stores across a range of activities from 0–100% and water temperatures ranging from 18.7 to 30 °C, the range of water temperatures encountered by free-ranging olive ridleys (McMahon et al. 2007; Chambault et al. 2016).

Stress indicators

As an indicator of physiological stress levels in individuals, we measured serum corticosterone and plasma glucose and lactate concentrations. Corticosterone was measured in duplicate using a commercially available ELISA (Cayman Chemical, Ann Arbor, MI, USA; kit #500655). This assay platform was validated for use in olive ridley sea turtles. Diluted samples showed parallelism to the standard curve, linearity of measured concentrations ($\beta = 1.04$, $r^2 = 0.99$) and accuracy of recovered standards ($1.05 \pm 0.04\%$). Intra-assay cv% was 4.71%. Glucose and lactate were measured in duplicate using a YSI-2300 STAT glucose/lactate autoanalyzer.

Statistical analysis

Statistical analysis was performed using JMP Pro 14 (SAS Institute, USA). Variables of interest were compared between the arribada and solitary nesting samples using two-sample *t* tests with pooled or unequal variance. Unequal variance was assessed using Levene's test. Relationships to body mass were assessed using simple linear regression. Statistical significance was considered at $p = 0.05$.

Results

Blood O_2 stores

Mean values for olive ridleys' mass, Hb, Hct, MCHC, PV and BV in wild nesting solitary females ($n = 10$), wild nesting arribada females ($n = 13$) are presented in Table 1. Blood Hb did not vary between nesting strategies ($p = 0.14$) but was more variable during the arribada period (Levene's test, $p = 0.03$). Hct was similar during the 2 nesting strategies ($p = 0.38$). The variation in Hb content was unrelated

Table 1 Values from arribada nesting, solitary nesting and their averages

	Arribada nesting	Solitary nesting	Average	Range
Mass (kg)	31.5 ± 1.3	29.6 ± 0.9	30.7 ± 0.7	22.3–40.0
Hb (g dL^{-1})	8.9 ± 0.5	9.7 ± 0.2	9.2 ± 0.3	6.0–12.9
Hct (%)	30.6 ± 2.4	32.9 ± 0.8	31.6 ± 1.4	18–50
MCHC (g dL^{-1})	0.31 ± 0.03	0.30 ± 0.01	0.31 ± 0.01	0.18–0.43
PV (mL)	1768.1 ± 44.8*	1506.5 ± 43.6	1654.4 ± 41.5	
PV (mL 100 g^{-1})	5.8 ± 0.1	5.1 ± 0.1	5.4 ± 0.4	4.4–6.6
BV (mL)	2593.3 ± 121.8*	2247.9 ± 68.7	2443.1 ± 82.12	
BV (mL 100 g^{-1})	8.4 ± 0.1	7.6 ± 0.1	8.1 ± 0.1	5.8–11.4
Blood O_2 (mL kg^{-1})	7.7 ± 0.6	7.8 ± 0.3	7.7 ± 0.4	3.6–10.8

Values presented are least square means ± standard error mean. Mass (kg)

Hb hemoglobin, *Hct* hematocrit, *MCHC* mean corpuscular hemoglobin concentration, *PV* plasma volume, *BV* blood volume

*Denotes significant differences between the nesting strategies ($p < 0.05$)

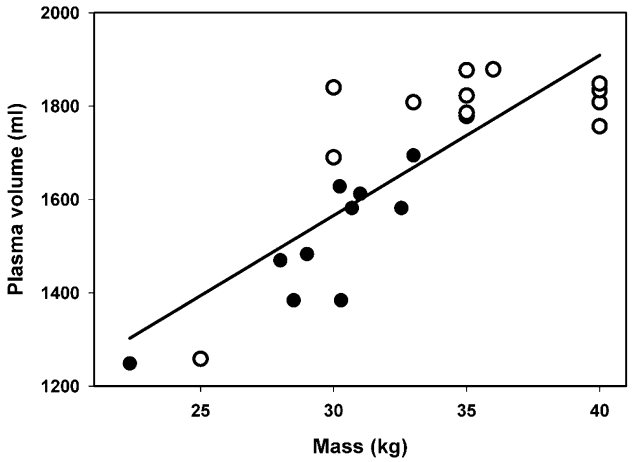

Fig. 2 Plasma volume increased with body mass. Open circles denote arribada nesting and closed circles denote solitary nesting ($r^2 = 0.31$, $p < 0.0001$). Plasma volume was calculated from dilution of Evan's Blue dye

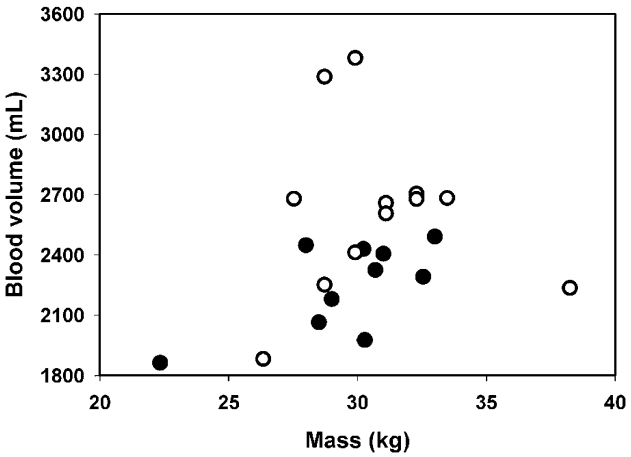

Fig. 3 Blood volume did not vary with body mass ($p = 0.35$). Open circles denote arribada nesting and closed circles denote solitary nesting. Blood volume was calculated as PV/ $[(100 - \text{Hct})^{-1}]$

to Hct ($p = 0.33$), due to individual differences in calculated MCHC. MCHC did not vary between nesting strategies ($p = 0.58$) but was more variable during the arribada period (Levene's test, $p = 0.03$).

Mean PV increased significantly with increased body mass ($PV = 28.3 * mass + 795.4$, $r^2 = 0.31$, $F_{1,21} = 9.37$ $p < 0.006$; Fig. 2). PV was 5.4 ± 0.4 (SD) % of body mass and this percentage was greater during the arribada period ($t = 3.04$, $df = 21$ $p = 0.006$). BV, calculated using PV and Hct was not related to body mass ($p = 0.35$; Fig. 3). BV was 8.1 ± 1.3 (SD) % of body mass and this percentage did not vary between nesting strategies ($p = 0.17$). Estimated mass specific blood O_2 stores were 7.74 ± 1.8 (SD) ml O_2 kg^{-1}. This value did not vary between nesting strategies ($p = 0.92$), but was more variable during the arribada period (Levene's test, $p = 0.008$; Fig. 4).

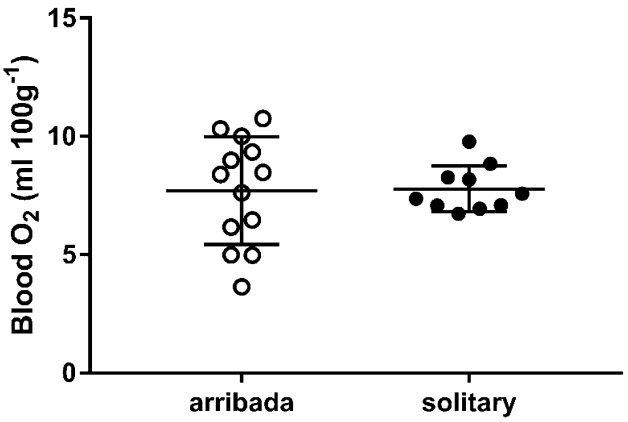

Fig. 4 Mass-specific blood O_2 stores calculated from plasma volume and Hct. Stores were more variable during the arribada nesting period (Levene's test, $p = 0.04$)

cADL and diving behavior

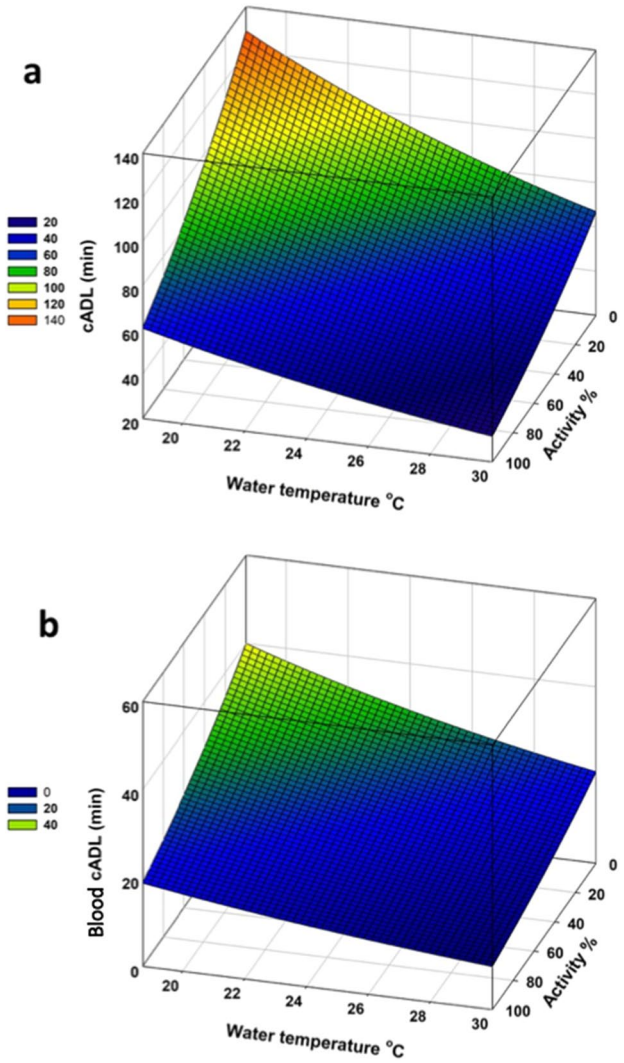

Fig. 5 Estimated cADL from total O_2 stores (**a**) and blood O_2 stores (**b**) at water temperatures ranging from 21.5–30.0 °C in 0.1 °C increments and 0–100% activity in 1% increments during the dive. Diving metabolic rate was estimated using Eq. 1 from Williams et al., 2019

cADL (min) from estimated total O_2 stores was calculated for water temperatures ranging 18.7–30.0 °C in 0.1 °C intervals (McMahon et al. 2007; Chambault et al. 2016) and for activity levels of 0–100% of the dive in 1% intervals (Kinoshita et al. 2018; Williams et al. 2019) (Fig. 5a). At 26 °C, the mean temperature value encountered by olive ridley's during their post-nesting migration (Chambault et al. 2016), cADL ranged from 40.5 ± 2.8 to 85.8 ± 5.9 min depending on activity levels. Blood O_2 stores represented 32.2 ± 5.2% of estimated total body O_2 stores. Thus, blood O_2 stores added 13.52 ± 2.9 to 27.9 ± 6.2 min to aerobic dive times in 26 °C water. In the coldest water (18.7 °C) and at rest (0% activity), blood O_2 stores added 42.7 ± 9.4 min to aerobic dive times (Fig. 5b).

Corticosterone and metabolites

Corticosterone concentrations were higher in arribada females (3.37 vs 2.69 ng/mL, $t = 3.14$, $p = 0.004$). Glucose concentrations were also higher in arribada females (4.56 vs 3.12 mmol/L, $t = 2.82$, $p = 0.01$). Lactate concentrations were highly variable among individuals but did not differ between the two breeding strategies (10.25 ± 4.54 mmol/L, $p = 0.27$).

Discussion

For a large sample of free-ranging nesting olive ridley sea turtles, blood volume was 8.1 ml 100 g^{-1} body mass and blood O_2 stores were 7.7 ml O_2 kg^{-1}. This represents ~ 32% of body O_2 stores when making standard assumptions on lung volume and muscle stores. These stores may represent a critical component of aerobic capacity on the longest and deepest dives exhibited by individuals of the species. Mass-specific blood volume was similar to that reported for leatherbacks (7.7%; Table 2) and was slightly higher than that reported previously for other chelonidae (6.6–6.7%; Thorson 1968). However, as previously reported, blood volumes

Table 2 Mean blood values for *L.* olivacea from this study and other species of sea turtles

Common name	Body mass (kg)	Hct (%)	Hb (g dL^{-1})	MCHC (Hb Hct^{-1})	Blood volume (mL 100 g^{-1})	Reference
Olive ridley	30.7	32	9.2	0.31	8.1	This study
Kemp's ridley	37.8	32	10.1	0.30	6.7	1,2,3
Loggerhead	35	41	9.8	0.24	6.7	1,4
Hawksbill	24	39	9.6	0.24	–	5
Green turtle	145	45	10.6	0.23	6.6	6,7
Leatherback	300	39	15.6	0.40	7.7	8,9

Hb values are expressed as (g/100 mL blood). Blood volume is presented as mL 100 g^{-1} (mass%) to facilitate comparison to published values

[1]Thorson 1968, [2]Davis 1991, [3]Marquez-M 1994, [4]Keller et al. 2004, [5]Muñoz-Pérez et al. 2017, [6]Wood et al. 1984, [7]Wood and Ebanks 1984, [8]Lutcavage et al. 1990, [9]Lutcavage et al. 1992

were not remarkable when compared to terrestrial species (Thorson 1968).

Mass-specific blood volume and blood O_2 stores varied widely among individuals, twofold and threefold, respectively. Plasma volume was relatively invariant and most of these differences were due to variation in hematocrit, Hb and associated MCHC content. This wide individual variation in blood O_2 stores emphasizes the importance of physiological state to aerobic capacity and the value of having a large sample size for assessing capacity of a species. Hb concentrations decreased weakly with body mass, but the drivers of widely varying hematocrit and most of the variation in Hb content of blood are unknown. Large sample size studies of sea turtle hematology yield similarly wide ranges of Hct among individuals (e.g. Lewbart et al. 2014; Rousselet et al. 2013; Wood and Ebanks 1984) and have demonstrated associations of Hct with stress, contaminant burdens, ectoparasites and tumor scores (Work and Balazs 1999; Keller et al. 2004; Stamper et al. 2005; García-Párraga et al. 2014). A significant correlation between Hct and the straight carapace length of sea turtles has been previously reported (Wood and Ebanks 1984) but we found no relationship to mass or size in our study.

We compiled published body mass, Hct, Hb content, BV and MCHC values for five species of sea turtles (Table 2). When compared to the other species, olive ridley sea turtle has the lowest Hct and Hb values. MCHC values were relatively consistent among other species, with the exception of the deep-diving *D. coriacea*. Closely related *L. kempii* and *L. olivacea* (Crawford et al. 2015) had highly similar MCHC values but showed inter-specific variation in blood volume consistent with their diving patterns, *L. kempii* exhibit the shallowest dives of all sea turtles, with the deepest recorded dive of 5.3 m (Sasso and Witzell 2006). *D. coriacea* is the species that had the largest values of mass-specific blood volume, consistent with their behavior as the deepest diver from all seven species (Lutcavage et al. 1992; Fossette et al. 2010). What was most surprising was the striking similarity on BV values between *D. coriacea* (7.7 ml 100 g^{-1}) *and L. olivacea* (7.6 ml 100 g^{-1}) sea turtles. This supports previous observations of *L. olivacea* as being the second deepest diver (420 m; Da Silva et al. 2011) of all species of sea turtles after *D. coriacea* (1280 m; López-Mendilaharsu et al. 2008). Blood O_2 stores may be especially critical if pulmonary shunts are activated as part of the dive response to avoid decompression injuries on the deepest and longest dives (Garcia Párraga et al. 2018). Interestingly, olive ridley sea turtles newly calculated blood O_2 stores are similar to those reported for the best avian divers, emperor penguins (*Aptenodytes forsteri*) (Ponganis et al. 2011), with blood stores representing 31% of body O_2 stores.

We found no significant difference in blood O_2 stores or associated variables between the two nesting strategies. A previous study found that Hct is higher in migrating turtles compared to nesting values (Yang et al. 2019). This difference was thought to be due to conditioning effects from longer, deeper dives during the migratory phase. We found no similar evidence of conditioning differences between arribada and solitary turtles despite differences in nesting strategies. It has been demonstrated that olive ridley populations differ widely in habitat use in that some individuals do not exhibit migrations and forage neriticly (Polovina et al. 2004; Chambault et al. 2016). We found no evidence of differences in physiological capacity that suggests consistent at-sea behavioral differences between the two strategies.

Although there were no significant differences in mean values of hematological variables between nesting strategies, several measured variables were more variable during the arribada nesting. Hct, Hb, MCHC, mass-specific PV and mass-specific blood O_2 stores were all more variable during arribada nesting. The underlying cause of this variability was not due to differences in mass and likely reflects physiological state. Some of these differences may reflect high levels of stress experienced by arribada nesters that might be a trade-off to the benefits of large aggregations. A recent study on transport stress in Kemp's ridley showed that the stress of transport increased variability in Hct in association with changes in corticosterone and glucose concentrations (Hunt et al. 2019). A study on different stress responses between solitary and nesting females found that arribada nesting turtles exhibited slower elevation of corticosterone in response to a stress test but did not compare baseline levels (Valverde et al. 1999). Our data suggest significantly higher corticosterone and glucose concentrations in arribada females. This finding was consistent with the idea that increased social stress during arribada may underlie the more highly variable Hct, but differences in the variability of other variables (e.g. MCHC and PV%) are less likely to have arisen from acute stress.

Based on measures of diving behavior in olive ridley sea turtles, blood O_2 stores are likely a critical component of aerobic respiration to many dives. Lung stores are similar among species, suggesting that diving differences might be most likely due to individual variation in blood O_2 stores rather than in muscle stores, given low myoglobin contents across species, ~ 4% of total body O_2 stores (Berkson 1966; Lutz and Bentley 1985; Stabenau and Heming 1994). Previous investigations reporting deep and long-dive behaviors exhibited by olive ridley sea turtles (Polovina et al. 2004; McMahon et al. 2007; Chambault et al. 2016) demonstrated that 77% of dive time represents active foraging behaviors, and that some dives exceed the presumed depths of lung collapse (~ 120 m, Berkson 1967). Thus, selection for increased blood O_2 stores may represent an important physiological adaptation for deep diving.

Our cADL estimates are limited by the paucity of information on olive ridleys. We lacked individual data on lung volume and muscle Mb content and estimated these values using data from other species. We also estimated Hb saturation at the beginning of the dive and arterial/venous differences using data from another species and used an assumed level of potential arterial desaturation. Despite this imprecision, our estimates clearly show the importance of blood O_2 stores to support the long-duration dives exhibited by the species. Our cADL estimates that incorporate blood stores are ~ 41 min for dives with high levels of activity and at mean water temperatures as reported for foraging dives. Blood O_2 stores provide ~ 14 min of that dive time and are likely necessary to support the diving behavior on many dives. Recorded average dive duration in olive ridleys is ~ 46 min and 40% of dives ranged between 30 and 50 min (Chambault et al. 2016). All dives are likely aerobic as their short and invariant post-dive surface times do not suggest that turtles are incurring a lactate debt. Use of pulmonary shunts to avoid decompression injury on some deep dives may further emphasize the importance of blood stores (Garcia Parraga et al. 2018).

Bycatch, the primary mortality threat to sea turtles, results in death from drowning or signs of decompression sickness in 43% of incidental captures (García-Párraga et al. 2014). The evidence for decompression injuries in incidentally captured turtles, emphasizes the importance of modulation of lung perfusion during diving in sea turtles and the potential for loss of access to lung stores during all or parts of dives. Individual and inter-specific variation in blood O_2 stores may be an important factor in avoiding drowning when captured in fishing nets.

The primary determinant of rates of O_2 use in diving sea turtles is activity levels (Williams et al. 2019). However, given high rates of activity on most foraging dives, water temperature is also an important modulator of diving metabolism. Changes in water temperature due to global warming can directly affect ectothermic body temperature and lower breath-hold capacity. Based on our calculations, a 1-degree change in mean water temperature reduces aerobic dive time by ~ 6% at high activity levels. Further, changes in thermal structure, currents and prey distribution in response to climate change may require substantial alterations to diving behavior (Hawkes et al. 2009). Individual and inter-specific variation in blood O_2 stores may be a crucial component of enabling behavioral plasticity in response to these changes. Given that this study only measured blood O_2 stores directly and used previously published values of lungs and muscles to cADL, direct measures of ADL in the future would be more accurate to determine the exact ADL on olive ridley sea turtles.

This first large sample study on blood O_2 stores in olive ridley sea turtles revealed that these stores represent a critical component of total body O_2 stores. Mass-specific blood volumes were similar to that reported for larger deeper diving leatherback sea turtles and appear to be essential to support the long-duration diving behavior reported for olive ridleys. The wide individual variation in blood O_2 stores has important implications for intra-specific variation in breath-hold ability. This variation was greater during the arribada nesting period due to increased variation in hematocrit, PV and MCHC. High blood O_2 stores may be a critical component of the behavioral plasticity needed to respond to changes in prey distribution, thermal structure and currents that result from climate change.

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Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by B. Gabriela Arango, Martha Harfush-Meléndez and Daniel E. Crocker. The first draft of the manuscript was written by B. Gabriela Arango and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data accessibility Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.nk98sf7s0 (Arango, Gabriela 2020).

Ethics approval Work was approved by the Sonoma State University IACUC and performed under collecting permit SGPA/DGVS/12915/16. Samples were exported/imported under CITES MX88143 and CITES 19US85728C/9.

Consent to participate/publication and availability of data/code Not applicable.

Conflict of interest The authors declare no conflict of interest.

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