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RESEARCH ARTICLE

Continuous arterial P_{O_2} profiles in unrestrained, undisturbed aquatic turtles during routine behaviors

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ABSTRACT

Mammals and birds maintain high arterial partial pressure of oxygen (P_{O_2}) values in order to preserve near-complete hemoglobin (Hb) oxygen (O_2) saturation. In diving mammals and birds, arterial O_2 follows a primarily monotonic decline and then recovers quickly after dives. In laboratory studies of submerged freshwater turtles, arterial O_2 depletion typically follows a similar pattern. However, in these studies, turtles were disturbed, frequently tethered to external equipment and confined either to small tanks or breathing holes. Aquatic turtles can alter cardiac shunting patterns, which will affect arterial P_{O_2} values. Consequently, little is known about arterial O_2 regulation and use in undisturbed turtles. We conducted the first study to continuously measure arterial P_{O_2} using implanted microelectrodes and a backpack logger in undisturbed red-eared sliders during routine activities. Arterial P_{O_2} profiles during submergences varied dramatically, with no consistent patterns. Arterial P_{O_2} was also lower than previously reported during all activities, with values rarely above 50 mmHg (85% Hb saturation). There was no difference in mean P_{O_2} between five different activities: submerged resting, swimming, basking, resting at the surface and when a person was present. These results suggest significant cardiac shunting occurs during routine activities as well as submergences. However, the lack of relationship between P_{O_2} and any activity suggests that cardiac shunts are not regulated to maintain high arterial P_{O_2} values. These data support the idea that cardiac shunting is the passive by-product of regulation of vascular resistances by the autonomic nervous system.

KEY WORDS: Turtle, Arterial P_{O_2}, *Trachemys scripta*, Cardiac shunt, Blood oxygen depletion, Diving

INTRODUCTION

Across air-breathing vertebrates, there is long-standing acceptance that oxygen (O_2) levels are regulated to meet metabolic demands and arterial values are kept high during almost all activities. Mammals and birds maintain a narrow range of arterial partial pressure of oxygen (P_{O_2}) values in order to preserve near-complete hemoglobin (Hb) oxygen saturation over a wide range of metabolic demands (i.e. from rest to vigorous activity). As arterial blood gases are tightly coupled with lung gases, arterial P_{O_2} values are typically near 100 mmHg and, during activity, maintained by appropriate adjustments in pulmonary ventilation. High Hb saturation and regulation of P_{O_2} to meet metabolic demands is believed to be true in reptiles as well. However, this has not been experimentally established. Aquatic turtles, with their low metabolic demands, hypoxemia tolerance and cardiac shunting capacity, may not have the same O_2 regulatory requirements as mammals and birds.

In terrestrial mammals working at the maximum rate of O_2 uptake ($\dot{V}_{O_2,max}$), high arterial P_{O_2} values are still maintained and hypoxemia rarely occurs even during heavy exercise in steady state (Dempsey et al., 1984; Taylor et al., 1987). In contrast, a decrease in O_2 is inevitable during diving. The duration of dives in marine mammals and birds is primarily a function of the magnitude of O_2 stores, the rate these stores are depleted and the tolerance the divers have to low O_2 (Butler and Jones, 1997; Kooyman and Ponganis, 1998). Because very low arterial P_{O_2} values are required for all O_2 molecules to dissociate from Hb, extreme hypoxemic tolerance allows for more complete use of the blood O_2 store during dives. Diving seals and penguins that allow arterial P_{O_2} values to fall below 30 mmHg on a regular basis use a much larger portion of the blood O_2 store than can be accessed by other, less hypoxemic-tolerant animals (Meir et al., 2009; Ponganis et al., 2011, 2009). However, these marine animals maintain high arterial P_{O_2} values prior to hypoxic events (diving) and O_2 levels are quickly restored after a dive (Kooyman et al., 1980; Meir et al., 2009; Ponganis et al., 2009; Qvist et al., 1986). Typical arterial O_2 depletion patterns in seals and penguins are characterized by an initial increase in P_{O_2}, followed by a slow, steady and monotonic decline until the ascent, when the depletion rate tends to increase (Meir et al., 2009; Ponganis et al., 2011, 2009, 2007).

Many freshwater turtles are extremely hypoxemic tolerant, surviving for long periods under hypoxic or anoxic conditions. Unlike mammals and birds, there is no tight coupling of lung and arterial blood gases in reptiles because of the incomplete separation of the pulmonary and systemic circulations, and arterial blood gases can be altered by changes in either ventilation or cardiac shunting patterns. Shunting can occur from differences in pulmonary and systemic vascular resistance (Hicks, 1994; Hicks and Comeau, 1994; Hicks and Wang, 1998; Wang and Hicks, 1996b). In right to left (R–L) shunts, which increase during apneas, oxygen-depleted blood bypasses the pulmonary circulation and returns to the systemic circulation. Increases or decreases in the degree of R–L shunting will immediately alter admixture and, thus, arterial P_{O_2} can change rapidly despite stable ventilation (Hicks and Wang, 1996; Wang et al., 1997; Wang and Hicks, 1996b). However, in past laboratory studies, most blood O_2 depletion patterns of submerged freshwater turtles (e.g. *Trachemys scripta*, *Chelodina longicollis*) are similar to those of diving mammals and birds, in which P_{O_2} starts high, depletes throughout a dive and then recovers upon surfacing. Some variation on this pattern has been observed, including positive and negative oscillations in arterial P_{O_2} values within dives (Burggren and Shelton, 1979; Burggren et al., 1989). Past studies

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List of symbols and abbreviations

Hb	hemoglobin
L–R	left to right
O_2	oxygen
P_{CO_2}	partial pressure of carbon dioxide
P_{O_2}	partial pressure of oxygen
R–L	right to left
TDR	time-depth recorder
$\dot{V}_{O_2,max}$	maximal oxygen consumption

investigating arterial O_2 levels have been on anesthetized turtles (Crossley et al., 1998; Hicks and Wang, 1999; Platzack and Hicks, 2001) or turtles in small, shallow tanks with a restricted (7–10 cm diameter) breathing hole and tethered to leads or catheters for serial or continuous blood sampling (Burggren and Shelton, 1979; Burggren et al., 1989; Wang and Hicks, 1996a, 2008; White et al., 1989). These experiments do not closely replicate natural behavior and disturbances may affect both behavioral and physiological responses in reptiles (Gaunt and Gans, 1969).

R–L shunts are reduced or even reversed during ventilation (Burggren and Shelton, 1979; Shelton and Burggren, 1976; Wang and Hicks, 1996a; White et al., 1989; White and Ross, 1966). If this change in R–L shunting patterns holds true in undisturbed turtles, when turtles are at the surface or out of the water arterial P_{O_2} should more closely reflect lung P_{O_2}, similar to mammals and birds. Although numerous laboratory studies have investigated the physiological adaptations underlying freshwater turtles' tolerance to low O_2 environments, few studies have investigated O_2 use in turtles during non-submerged routine activities. As a result, patterns of arterial O_2 use in minimally disturbed turtles, whether submerged or at the surface, are completely unknown.

We undertook to make the first continuous arterial P_{O_2} measurements in undisturbed, untethered red-eared sliders [*Trachemys scripta elegans* (Weid 1839)] free to dive or not dive in large tanks over an extended period. Our goals were to measure arterial P_{O_2} using implanted microelectrodes (tip diameter=0.5 mm) and a waterproof backpack logger during different activities, including resting submergences, swimming, basking, resting at the surface and when a person was present. We predicted that when turtles were at the surface or out of the water, arterial P_{O_2} values will vary but will be significantly higher than during submergences. With their anoxia tolerance and capacity to R–L shunt, we also predicted that P_{O_2} during submergences would vary more than observed in marine mammals and birds, but the final P_{O_2} would be negatively related to duration of submergences, particularly after long submergences.

MATERIALS AND METHODS

Animals and instrumentation

Six adult female red-eared sliders (1546±145 g) were collected in northeastern Texas and shipped to the University of California Irvine, where they were housed in a room maintained at 25°C with a 12 h:12 h light:dark cycle. Turtles were kept in 800 litre (76×180×66 cm) fiberglass tanks two-thirds filled with 25°C water. Water was continuously filtered and temperature was maintained by an in-line heater with a thermostat. Turtles could freely swim, rest at the bottom or at the surface hanging onto platform ramps, or bask on a platform (76×60 cm) heated by an overhead heat lamp. The surface of the large tank was never covered so turtles always had unrestricted access to the surface during the experimental period. Turtles were fed turtle pellets (Tetra ReptoMin Floating Food Sticks, Spectrum Brands, Blacksburg, VA, USA), carrots and lettuce twice a week.

Surgery

After an acclimation period of at least 4 weeks, turtles underwent surgery to implant P_{O_2} electrodes (Licox, C1.1 Revoxode; Integra Life Sciences, Plainsboro, NJ, USA). Prior to implantation, P_{O_2} electrodes were pretreated with TDMAC heparin complex (Polysciences, Washington, PA, USA). Turtles were anesthetized by placing them in a sealed container with gauze soaked in isoflurane and then intubated with an endotracheal tube. Turtles were ventilated using an artificial ventilator (SAR-830; CWE, Ardmore, PA, USA) with integrated isoflurane vaporizer (Dräger, Lübeck, FRG) at a rate of 8–15 breaths min^{-1} (Wang and Hicks, 2008) with 4–5% isoflurane and room air. Isoflurane was reduced to 1–2% after turtles no longer responded to a pinch reflex test or corneal reflex test. Utilizing a sterile (betadyne) preparation, the right carotid artery was isolated and gently separated from the vagus nerve. The artery was cut and the P_{O_2} electrode was inserted and advanced 6 to 10 cm toward the heart. Post-experiment dissections confirmed P_{O_2} electrodes were in the common carotid or right aortic arch. The vessel was tied off and the incision closed with 3-0 silk. Surgical adhesive was also applied to the incision site (Vet-Bond, 3M, St Paul, MN, USA). The electrode was secured with additional sutures and the electrode cable was attached to the carapace with waterproof Tesa tape (Beiersdorf AG, Hamburg, Germany) and glue (Loctite 401, Henkel, Westlake, OH, USA). The P_{O_2} electrode was connected to a microprocessor logger (UUB/3-EPTb, UFI, Morro Bay, CA, USA) with a custom-made plastic waterproof housing (entire package: 6.3×3×2 cm, 99 g in air), which was then epoxied to the center of the carapace. The logger was programmed to record P_{O_2} data continuously at a frequency of 1 Hz. A time–depth recorder (TDR; DST-milli, Star-Oddi, Gardabaer, Iceland; 4×1.3 cm, 9 g in air; 5 g in water, 0.33 to 1 Hz sampling rate, resolution: ±0.6 cm, <0.1°C) was attached to the left anterior carapace and recorded turtle submergence behavior and ambient temperature. Turtles were returned to experimental tanks after overnight recovery from surgery. Visual observations confirmed that post-procedure turtles adjusted to the loggers such that they could easily swim, dive, rest on the bottom or float at the surface. All procedures were approved by the University of California Irvine Institutional Animal Care and Use Committee (no. 2011-2995).

Data processing

After experiments were completed, the P_{O_2} logger and TDR were removed and the data were downloaded. Collected logger data were converted to P_{O_2} as previously described (Knower Stockard et al., 2005) and then plotted in a graphics program (Origin 2015, OriginLab Corporation, Northampton, MA, USA). Continuous temperature profiles from the TDR demonstrated mean temperature was 25.4±0.8°C. No temperature correction was made to P_{O_2} values because external temperature, as measured on the carapace, varied very little during the experimental period, with >95% of measured temperature within two standard deviations of the mean (25.4°C).

P_{O_2} electrodes

To continuously measure arterial P_{O_2}, commercially available electrodes (Licox, C1.1 Revoxode; Integra Life Sciences, Plainsboro, NJ, USA) were used. The 90% response time to changes in P_{O_2} for these electrodes is 60 s (Meir et al., 2009; Knower Stockard et al., 2005). P_{O_2} electrodes were calibrated as

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previously described except at 25°C (Knower Stockard et al., 2005). Although prior studies demonstrate no significant drift in these electrodes in mammals and birds (Meir et al., 2009; Knower Stockard et al., 2005), a post-experiment calibration was performed to assess drift in electrode output at 25°C.

After a 24-h recovery period, turtles were kept in tanks and left undisturbed except as noted. Investigator presence in the experimental room was minimized and each occurrence was documented. Turtle behavior was recorded continuously using a video surveillance system consisting of surveillance recording software (SecuritySpy, Ben Software, London, UK) and two network cameras (Panasonic BB-HCM511A & BLC140A, Panasonic, Osaka, Japan) placed above the tank. Video observations were recorded through a computer (Mac Mini, Apple, Cupertino, CA, USA) and stored on external hard drives. Turtle behavior was assessed by reviewing video data and dividing behavior into five categories: (1) submerged or resting on the bottom of the tank; (2) stationary at the surface, often hanging onto a floating ramp; (3) on top of the basking platform; (4) swimming submerged or at the surface; and (5) when a person was present in the room. Instances in which a turtle was not in view were omitted from the behavioral analysis. Behavioral data for each turtle were confirmed by plotting video data with TDR data. Any conflicts between the two data sets were resolved by rechecking the video behavior and reconfirming the behavior with TDR data. Submergences were manually extracted from the submerged data using the combined TDR and behavior profiles. During submergences, turtles primarily rested on the bottom. Submergences with significant swimming were categorized as swimming and not included as submergences in the analysis.

Statistics

Mean and range (maximum to minimum) of P_{O_2} values were obtained for each behavioral category during the 48-h period. Differences between mean P_{O_2} in each behavioral category were assessed with ANOVA and *post hoc* pairwise comparisons using Tukey's tests in Origin. Instances in which a behavior occurred for less than 15 min during the 48-h period were omitted. For each turtle, mean values were calculated for P_{O_2} parameters for all submergences in excess of 2 min, including mean, maximum, minimum, initial, and final P_{O_2}. The number of long (>20 min) and short (between 2 and 20 min) submergences, submergence duration and total change in P_{O_2} (from start of submergence to end) were also calculated. Differences between mean and initial P_{O_2} values during submergences were evaluated using a paired *t*-test in Origin. Statistics to assess the relationship between P_{O_2} characteristics (initial P_{O_2}, maximum P_{O_2}, final P_{O_2} and minimum P_{O_2}) and submergence duration were run in R, version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Because of repeated sampling, analyses were done using linear mixed effects models (package: lme4; Bates et al., 2015) with individual turtles as random effects and submergence duration as the fixed effect. In light of the high variability between individual turtles, we ran both random intercept and random slope models. Model selection was based on AIC scores. Reported *P*-values were calculated using a likelihood ratio test of the full model and the null model for both random intercept models and random slope models. Marginal and conditional r^2 values were calculated to determine goodness of fits using the methods previously described for random intercept models (Nakagawa and Schielzeth, 2013) and random slope models (Johnson, 2014) using the MuMIn package (https://CRAN.R-project.org/package=MuMIn). Model descriptions and results from mixed effect model analyses are reported in Table S1. Model assumptions of homogeneity and normality were evaluated and confirmed by examination of residuals. P_{O_2} data are expressed as measured in mmHg (7.5 mmHg=1 kPa). Reported Hb saturation values were calculated from P_{O_2} data using the Hill equation [$Y_{O_2}=(P_{O_2})^n/(P_{50})^n+(P_{O_2})^n$], a Hill coefficient ($n$) of 1.71 and a P_{50} value of 21 mmHg (Burggren et al., 1977). Arterial O_2 content was calculated from the equation: $Ca_{O_2}=(S_{a,O_2}\times Hb\times 1.36)+(0.0031\times P_{O_2})$, where S_{a,O_2} is arterial Hb saturation and Hb is assumed to be 8.0 g dl^{-1} (O'Rourke and Jenkins, 2008). Means are expressed ±s.d.

RESULTS

Arterial P_{O_2} during different activities

Arterial P_{O_2} showed no consistent values or ranges during any of the five different activities (Fig. 1). Values for each activity ranged from 4 mmHg to above 99 mmHg with significant variation among individual turtles (Table 1). When turtles were at the surface or out of the water basking, P_{O_2} was often surprisingly low (Table 1, Fig. 1). Over the 48-h period, P_{O_2} values in five of the six turtles dropped below 20 mmHg while swimming, submerged or at the surface (Table 1, Fig. 1A). There was no difference in mean P_{O_2} between five different activities (one-way ANOVA, F=0.37, P=0.82, d.f.=4; Fig. 2). Although this experiment focused on a 48-h period in which behavior was recorded, arterial P_{O_2} also varied over longer durations (Fig. 3).

Submergences

P_{O_2} and TDR submergence data were obtained from six turtles during 48-h periods (Table 2). A total of 529 submergences of 2 min or greater were analyzed. Of those, 168 were longer than 20 min and 46 went beyond 40 min. All turtles completed submergences longer than 20 min and five turtles had submergences over 40 min in duration. The longest submergence was just over 83 min by turtle 35 (Fig. 1C).

Initial arterial P_{O_2} values

Although there was a wide range in arterial P_{O_2} values, turtles did not start submergences with a high P_{O_2}. Only a few dives began with an arterial P_{O_2} above 60 mmHg (Fig. 4A). Initial P_{O_2} was not related to submergence duration (P>0.1). Further, there was no significant difference between individual turtle means for initial P_{O_2} and mean P_{O_2} (t=0.20, d.f.=5, P=0.85; Table 2). Initial P_{O_2} was rarely the highest P_{O_2} value during submergences (Table 2). In over half of all submergences longer than 20 min, maximum P_{O_2} occurred during the first 10% of the submergence (Fig. 4C). Despite this trend, there was no statistically significant relationship between maximum P_{O_2} and duration (P>0.5; Table S1).

Arterial P_{O_2} depletion patterns

P_{O_2} values during submergences did not follow a consistent pattern. P_{O_2} profiles varied among both short and long submergences: increasing, decreasing, increasing and decreasing, or changing very little (Figs 5 and 6). Final P_{O_2} was often higher than initial P_{O_2} (Table 2, Fig. 6). This occurred even in submergences longer than 20 min, where 64 of 168 submergences ended with a higher P_{O_2} than at the start of the submergence (Figs 5B,C and 7). Despite this high variability, there were some general trends in P_{O_2}. As described above, maximum P_{O_2} values often occurred near the beginning of submergences and P_{O_2} regularly dropped to minimum values during the final 10% of submergences (Fig. 4C).

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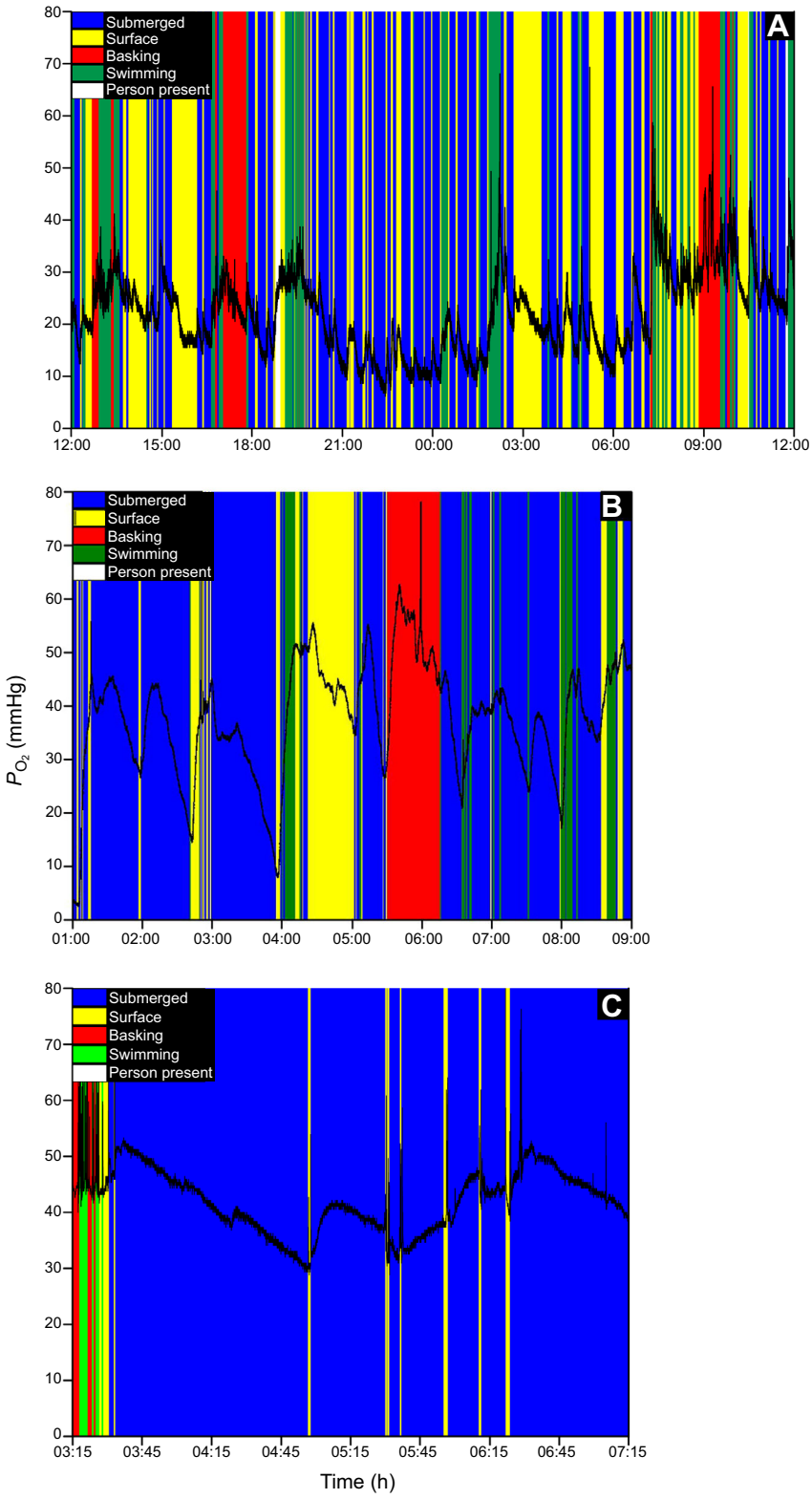

Fig. 1. Continuous arterial P_{O_2} measurement during different activities in *Trachemys scripta*. Data are for (A) 1 day in turtle 30 (12:00 h on 10 January 2015 to 12:00 h on 11 January 2015), (B) 8 h in turtle 32 (01:00 to 09:00 h on 26 November 2014) and (C) 4 h in turtle 35 (03:15 to 07:15 h on 18 January 2015). Each activity is represented by a different color and arterial P_{O_2} is represented by the black line. Note that the longest submergence (83 min) of the study is depicted in C.

Final arterial P_{O_2} values

Final P_{O_2} values also varied widely between submergences and turtles (Fig. 4B). Among individual turtles, mean final P_{O_2} ranged from 21 to 44 mmHg. Despite the number of submergences with a negative P_{O_2} change (Fig. 7), final P_{O_2} had a minor statistically significant relationship with submergence duration (X^2=4.55, marginal r^2=0.07, P=0.03; Fig. 4C, Table S1). The very strong effect of individual turtle is illustrated by the high conditional r^2 values (conditional r^2=0.6; Table S1). Final P_{O_2} was rarely the minimum P_{O_2} value as P_{O_2} often increased at the end of dives when the turtle was ascending to the surface (Fig. 6). Minimum P_{O_2} occurred within the last 10% of the submergence (Fig. 4C) in over half of submergences longer than 20 min, and was also marginally related to submergence duration (X^2=27.3, marginal r^2=0.06, P<0.001; Table S1).

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Table 1. Mean arterial P_{O_2} and range (lowest to highest values) for individual turtles during different activities over a 48-h period

Turtle ID	Submerged (mmHg)	Surface (mmHg)	Basking (mmHg)	Swimming (mmHg)	Person present (mmHg)
26	39.3 (2–69)	30.7 (4–61)	*	37.3 (2–69)	27.3 (4–69)
28	32.8 (15–66)	35.0 (17–65)	39.8 (24–83)	36.9 (17–88)	36.6 (27–61)
29	43.2 (9–78)	53.6 (10–77)	40.1 (12–74)	38.9 (7–71)	40.3 (12–57)
30	21.6 (6–53)	22.6 (7–69)	29.2 (15–66)	28.2 (8–86)	30.7 (20–51)
32	34.1 (3–65)	38.0 (3–65)	48.9 (28–78)	40.7 (11–57)	32.2 (11–51)
35	42.8 (25–92)	40.9 (22–99)	47.6 (27–105)	48.2 (28–105)	50.5 (35–104)
Grand mean	35.6±8.1	36.8±10.4	41.1±7.9	38.4±6.5	36.3±8.3

*Duration of activity <15 min during the 48-h period.

Arterial Hb saturation and O_2 content

Hb saturation values are estimated because not all determinants of the O_2–Hb dissociation curve were measured, e.g. pH and P_{CO_2}. Using these estimates, Hb saturation profiles of the periods shown in Fig. 1 and of the submergences shown in Figs 5 and 6 were made (Figs S1–S3). Estimates of arterial Hb saturation while turtles were at the surface or basking varied from 54% to 83%. Mean Hb saturation values and ranges during all activities were also estimated (Table S2). Hb saturation values were rarely above 85%. For submergences, estimated mean initial Hb saturation values ranged from 56% to 82% and final values were between 52% and 79%. Arterial O_2 content, calculated from the mean arterial P_{O_2} in Table 2 and estimated Hb saturation values, ranged from 6.2 to 9.1 ml O_2 100 ml^{-1} at the beginning of dives and from 5.7 to 8.8 ml O_2 100 ml^{-1} at the end of dives.

P_{O_2} electrodes

Although not all electrodes could be tested owing to breakage, a comparison of pre- and post-deployment calibrations at 25°C was conducted for the P_{O_2} electrode implanted in turtle 32. Arterial P_{O_2} profiles using both calibrations demonstrate no significant change in P_{O_2} values, even with several months between calibrations (Fig. S4). Although some minor drift occurred between the two calibrations, arterial P_{O_2} values changed less than 2 mmHg.

DISCUSSION

This first study to continuously measure arterial P_{O_2} in undisturbed and untethered aquatic turtles over days revealed that, unlike marine mammals and birds, aquatic turtles do not tightly regulate arterial O_2 levels. Although turtles were under identical conditions for days at a time with unfettered ability to move about the tanks, arterial P_{O_2} levels varied widely during the same activities. Contrary to predictions, P_{O_2} values remained low while turtles were at the surface or out of the water. In addition, there was no consistent arterial P_{O_2} pattern during submergences.

Lack of tight O_2 regulation during routine activities

Individual differences over the 48-h period are apparent in all activities, including during both submergences and non-submerged activities (Table 1). Further, the variation in arterial P_{O_2} values during routine activities is even more evident when examining arterial P_{O_2} over a 1-week period (Fig. 3). The extremely variable arterial P_{O_2} values are in contrast to marine mammals and birds, which generally maintain high, stable arterial P_{O_2} values except when diving. We suggest that *T. scripta*, because of several chelonian attributes including cardiac shunting, low metabolism, high hypoxia tolerance and a large lung O_2 store, do not need to maintain stable, high arterial P_{O_2} values or tightly regulate blood O_2.

Cardiac shunting

First, cardiac shunting may underlie these variable values. The ventricle of the three-chambered turtle heart is partially divided by a poorly developed muscular ridge into the cavum pulmonale and the cavum venosum/cavum arteriosum (Hicks and Wang, 2004). As a result of this anatomical feature, when pulmonary vascular resistance is elevated relative to the vascular resistance in the systemic circulation, a R–L shunt prevails (Hicks, 1994; Hicks and

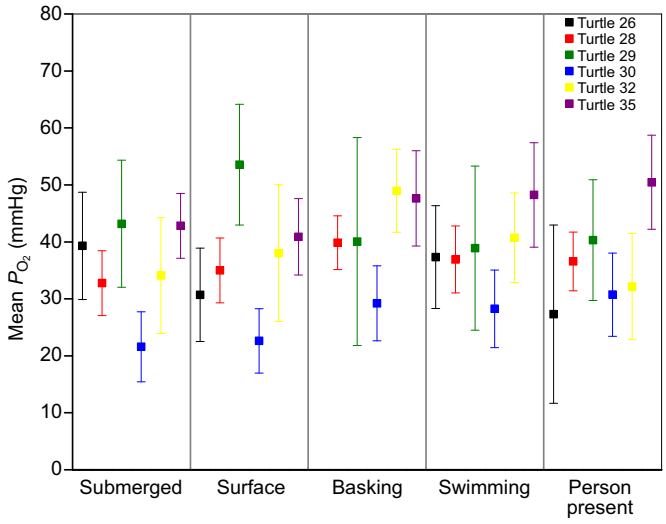

Fig. 2. Mean ±s.d. P_{O_2} for individual turtles during each activity. *N*=6 for all activities except basking (*N*=5).

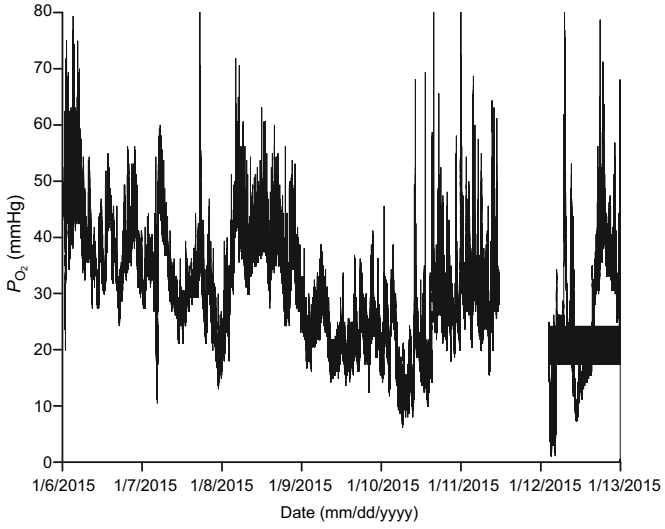

Fig. 3. One-week profile of continuous arterial P_{O_2} measurement in one turtle. Note that the range of arterial P_{O_2} changed over different days (e.g. 6–7 January, 25–55 mmHg versus 9–10 January, 10–40 mmHg).

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Table 2. Individual turtle mass, number, duration and mean, maximum, minimum, initial, final, mean and net change (from initial to final) arterial P_{O_2} for all submergences 2 min or longer in duration

Turtle ID	Mass (mg)	*N*	*N* >20 min	Duration (min)	Maximum P_{O_2} (mmHg)	Minimum P_{O_2} (mmHg)	Initial P_{O_2} (mmHg)	Final P_{O_2} (mmHg)	Mean P_{O_2} (mmHg)	P_{O_2} change (mmHg)
26	1627	89	51	24.2±15	46.7±11	33.0±11	37.1±11	37.8±11	38.2±10	0.8±8
				(2–63)	(17–69)	(3–60)	(3–60)	(3–56)	(10–55)	((–)29–29)
28	1397	96	45	19.9±14	45.3±7	29.1±6	35.3±6	32.6±6	34.0±55	2.7±8
				(2–61)	(28–67)	(15–44)	(17–50)	(15–49)	(24–47)	((–)14–34)
29	1680	38	22	23.2±16	49.4±12	34.2±11	39.9±12	39.4±11	41.4±11	0.5±8
				(2–52)	(17–78)	(15–64)	(16–71)	(16–65)	(16–70)	((–)18–21)
32	1496	70	16	13.7±14	43.0±9	29.5±12	37.1±10	32.9±12	36.0±9	4.1±13
				(2–56)	(12–65)	(2–52)	(3–62)	(3–62)	(6–36)	((–)24–43)
30	1372	146	6	11.0±5	27.4±7	19.3±6	23.3±6	20.8±6	22.3±6	2.5±3
				(2–26)	(14–53)	(6–37)	(11–41)	(9–37)	(10–39)	((–)7–13)
35	1705	90	28	17.1±15	64.0±15	37.6±5	48.9±8	43.9±7	43.0±5	–1.1±8
				(2–83)	(39–92)	(25–50)	(26–70)	(30–72)	(31–55)	((–)20–31)
Grand mean	1546±145	88±35	28±17	18.2±5	46.0±12	30±6	37±8	35±8	36±7	1.6±1.9

N, number of submergences. Mean±s.d. is given in the first row and range is shown in parentheses in the second row for duration and P_{O_2} parameters.

Comeau, 1994; Hicks and Wang, 1998; Wang and Hicks, 1996b). Under these conditions, systemic venous blood bypasses the pulmonary circulation and reenters the systemic circulation, resulting in overall lower arterial P_{O_2} values. In addition, the lower pulmonary blood flow reduces the transfer of O_2 from the lungs to the pulmonary capillaries.

The highly variable and overall lower arterial P_{O_2} values in the present study are likely the result of increased cardiac shunting. Changes in the degree of R–L or L–R shunting would account for the variability in arterial P_{O_2} profiles within submergences (Wang and Hicks, 1996b). Although variation in cardiac shunting patterns has been observed in turtles during different behaviors (Krosniunas and Hicks, 2003), most past studies demonstrate that a R–L shunt prevails during apnea, while it is reduced or reversed during ventilation (Burggren and Shelton, 1979; Shelton and Burggren, 1976; Wang and Hicks, 1996a; White et al., 1989; White and Ross, 1966). The comprehensive nature of our study and less disturbed environment in which it was undertaken suggest additional changes in shunting patterns, producing less uniform gas exchange and resulting in highly variable and overall lower arterial P_{O_2}. Although studies demonstrating differences in degrees of cardiac shunting have focused on the biological significance of regulating shunting, no study has been able to establish a functional role. The lack of a relationship between arterial P_{O_2} values and specific activities suggest changes in levels of cardiac shunting are not predictable or related to specific turtle activities. Thus, although the increased changes in cardiac shunting patterns provides a mechanistic explanation of the observed low and variable values, the benefit, if any, of not maintaining high, stable arterial P_{O_2} remains unknown.

Low metabolism and high anoxia tolerance

Second, turtles are easily able to handle these low and inconsistent P_{O_2} values because of their naturally low metabolic rate and their high tolerance of low O_2 conditions. Their low metabolic rate reduces demand for O_2 and prolongs the time they can survive anoxic submergences (Warren et al., 2006). In addition to needing less O_2, freshwater turtles can endure submergences for weeks despite lactate levels as high as 90 to 200 mmol l^{-1} (Warren and Jackson, 2008; Warren et al., 2006). *Trachemys scripta* can survive submerged in 3°C normoxic water for up to 180 days and in 22°C anoxic water for over 20 h (Belkin, 1968; Warren et al., 2006). Freshwater turtles handle elevated lactate levels with no difficulty because of their high buffering capacity, including uptake of lactate in the shell and skeleton (Warren and Jackson, 2008). For example, despite a fall in arterial Hb saturation from 91% to 34% and a sixfold increase in blood lactate after vigorous activity, pH in red-eared sliders was only slightly lower, from a pH of 7.19 at rest to 7.17 (Gatten, 1975). Thus, these turtles can easily tolerate the variability of arterial P_{O_2} values observed in this study.

Large lung O_2 store

Finally, if an increase in arterial P_{O_2} is required, turtles can access the O_2 stored in the lungs. The turtle lung provides a large O_2 reservoir, accounting for over two-thirds of the total body O_2 store. Because gas exchange is not cut off in the shallow diving freshwater turtle, pulmonary and systemic blood flow can be increased or decreased during dives (Krosniunas and Hicks, 2003) to elevate arterial P_{O_2} without the turtle having to surface. The increases in arterial P_{O_2} observed during some submergences suggest this is occurring (see e.g. Fig. 5). Further, distinct lung and arterial O_2 profiles demonstrate that turtles may deplete the lung O_2 store in a non-uniform, intermittent manner through changes in cardiac shunting patterns during submergences (Burggren and Shelton, 1979).

The combination of the present results, freshwater turtles' low O_2 requirements, their high tolerance to anoxia and their ability to access lung O_2 during submergences suggest that these turtles do not need to tightly regulate arterial O_2 levels.

Tight regulation of O_2 – a disadvantage?

In turtles at a relatively constant body temperature, maintaining a high arterial P_{O_2} requires minimizing R–L cardiac shunting. However, precise regulation of cardiac shunting to maintain high blood O_2 levels may not be possible for freshwater turtles under all physiological states. For instance, during digestion or activity, the regulation of blood flow to various tissues and organs may influence the hemodynamic state, and the associated changes in cardiac shunting patterns and effects on blood O_2 levels are simply the passive consequences of these adjustments (Hicks, 2002). Alternatively, if blood O_2 levels needed to be tightly regulated, the lack of separation in the ventricle would mean regulation of vascular beds would be constrained by the effect on cardiac shunting, limiting the ability of the autonomic nervous system to respond to changing conditions. For example, peripheral vasodilation occurs during basking, resulting in the development of a large R–L shunt (Galli et al., 2004). Because a R–L shunt will lower arterial P_{O_2}, if arterial O_2 levels were tightly regulated, the ability of turtles to thermoregulate during basking would be constrained.

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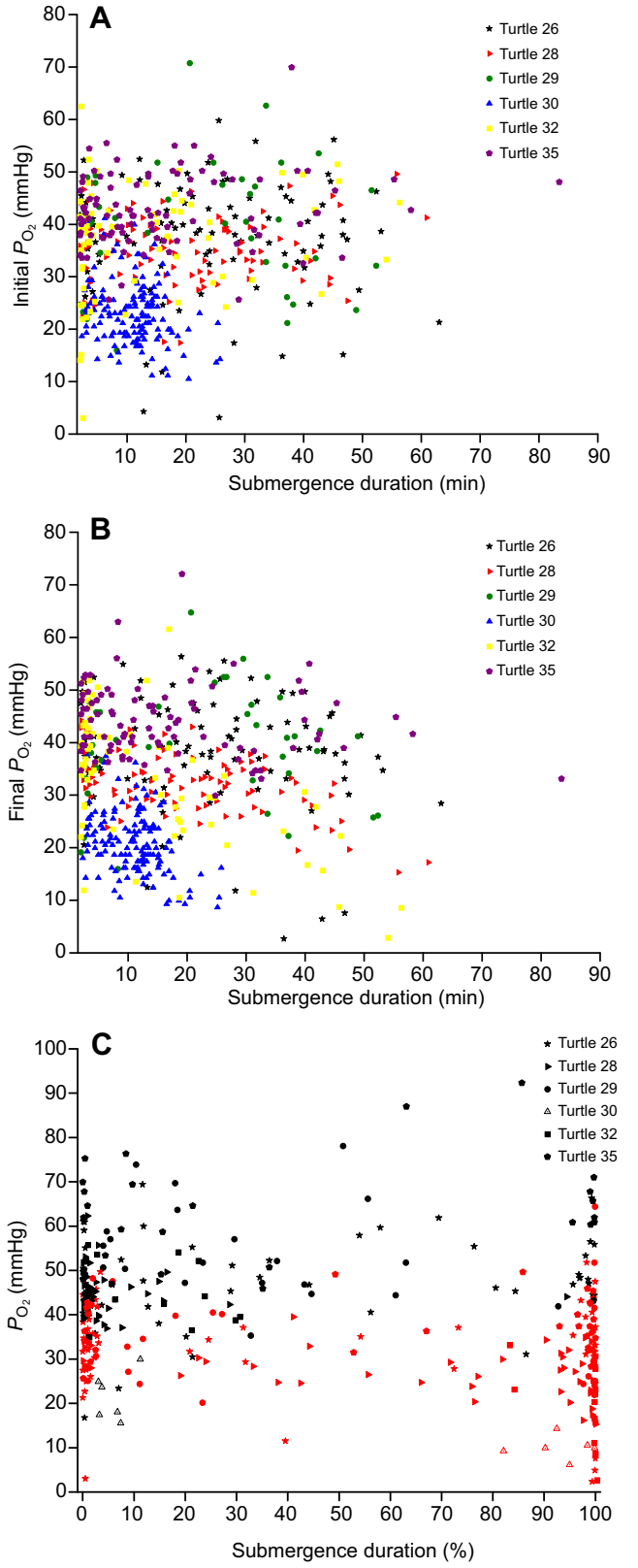

Fig. 4. Arterial P_{O_2} and submergence duration. (A) Initial P_{O_2} versus submergence duration and (B) final P_{O_2} versus submergence duration. Number of submergences=529. (C) Maximum (black symbols) and minimum (red symbols) P_{O_2} versus percent of submergence duration in submergences longer than 20 min. Note the concentration of maximum and minimum P_{O_2} values at the beginning or ending portion of submergences. Number of submergences=168.

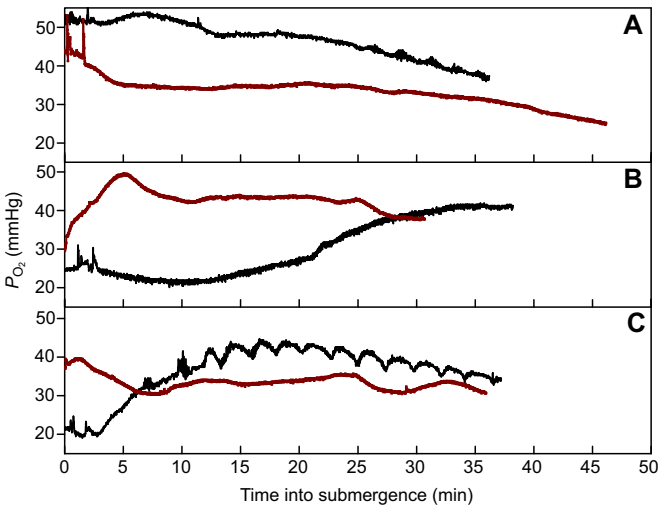

Fig. 5. Arterial P_{O_2} profiles in long submergences in two turtles. P_{O_2} profiles varied among submergences with P_{O_2} decreasing (A), increasing (B) and fluctuating up and down (C). Red profiles, turtle 28; black profiles, turtle 29.

Arterial P_{O_2} is not high during basking, at the surface or at the start of submergences

Arterial P_{O_2} values were not elevated when turtles were basking, at the surface or at the beginning of submergences (Tables 1 and 2). We had predicted that arterial P_{O_2} would be high at the surface because past studies found a reduction or reversal of R–L shunts during ventilatory periods. Further, because red-eared sliders immediately dive below the water's surface if disturbed,

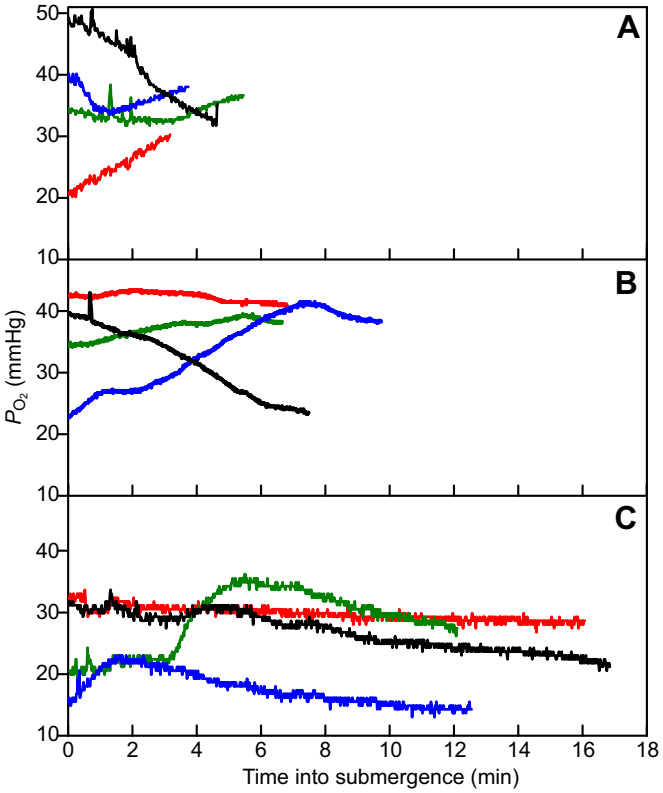

Fig. 6. Arterial P_{O_2} profiles in submergences of different durations. (A) Submergences of 2–6 min from turtle 29, (B) submergences of 6–11 min from turtle 32 and (C) submergences of 12–18 min from turtle 30.

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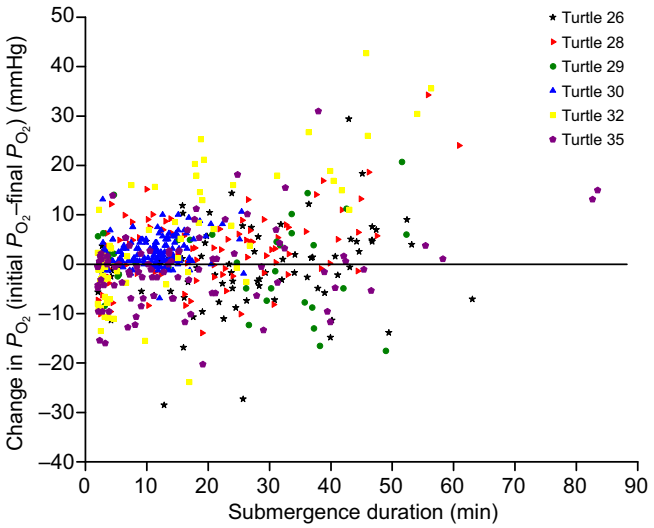

Fig. 7. Change in P_{O_2} from beginning to end of submergence versus submergence duration. Negative values indicate P_{O_2} at the end of a submergence was higher than initial P_{O_2}. Number of submergences=529.

maintenance of high arterial P_{O_2} values when they are out of the water or at the surface might therefore be beneficial. However, turtles typically began submergences near 37 mmHg (or at approximately 74% Hb saturation) and mean P_{O_2} values ranged from 23 to 54 mmHg (54% to 83% Hb saturation) when turtles were resting at the surface or basking. These values are lower than those found in past studies on both anesthetized and voluntarily diving turtles, where values ranged from 55 to 119 mmHg (Burggren and Shelton, 1979; Burggren et al., 1989; Crossley et al., 1998; Hicks and Wang, 1999; Platzack and Hicks, 2001), and suggest that R–L shunts continue to play a significant role during all ventilatory periods in undisturbed turtles. In short, *T. scripta* do not appear to anticipate or prepare for submergences by increasing initial P_{O_2} before a submergence (Table 1, Fig. 4A), and consistently begin submergences well below 90% Hb saturation.

No typical P_{O_2} pattern during submergences

Surprisingly, there were no typical arterial P_{O_2} patterns during submergences. Air-breathing animals must rely on the O_2 stored within the body during submergences, and there is a clear decrease in overall O_2 levels as the animal remains underwater. This reduction in O_2 is apparent in blood O_2 profiles of freely diving mammals and birds (McDonald and Ponganis, 2013; Meir et al., 2009; Ponganis et al., 2009, 2007). In contrast to our predictions, final arterial P_{O_2} values in the present study were not strongly related to submergence duration. Further, although some variation in arterial P_{O_2} values was predicted, the lack of typical arterial P_{O_2} profiles during submergences was not anticipated. In particular, the lack of a consistent decline in P_{O_2} during submergences was unexpected.

Final arterial P_{O_2} values and submergence duration

Final values for dives varied widely (3 to 72 mmHg; Table 2). Despite this variation, our prediction that final arterial P_{O_2} values would be related to dive duration was statistically correct in submergences of >2 min (P<0.001). However, with a marginal r^2 value of 0.07, the usefulness of this result to predict final P_{O_2} values is negligible (Table S1). We also predicted that this relationship would be stronger in longer dives. However, in submergences longer than 20 min, the marginal r^2 value for the best fit model was even lower (0.06). A visual examination of Fig. 4B showing final P_{O_2} and duration for all submergences also does not suggest a strong relationship. Further, there was very little difference between the grand mean final P_{O_2} (35 mmHg) and the grand mean initial P_{O_2} (37 mmHg) (Table 1). Rather, the more consequential interpretation of the statistical results is in the strong role that the individual turtle played in the relationship. A comparison of the marginal r^2 and conditional r^2 values demonstrates that, rather than the effect of duration, the strong influence of individual turtle effects explained much of the variation in the relationship between final P_{O_2} and duration. In fact, conditional r^2 values were high for most models (Table S1), emphasizing the high variability between individual turtles. Thus, these data and results do not suggest a biologically significant relationship between final or minimum P_{O_2} and dive duration; rather, they emphasize the high level of variation among and within individuals.

Arterial P_{O_2} profiles during submergences

In all turtles, arterial P_{O_2} profiles demonstrated increases, decreases, and both increases and decreases during submergences (Figs 1 and 2), but there was no typical P_{O_2} profile for any individual turtle or duration. These profiles are in contrast to the only other studies continuously measuring arterial P_{O_2} in undisturbed air-breathing animals (McDonald and Ponganis, 2012; Meir et al., 2009; Ponganis et al., 2009). In the diving emperor penguin, after an initial increase in air sac P_{O_2} owing to compression hyperoxia, air sac P_{O_2} declines throughout the dive, demonstrating maintenance of gas exchange with the lungs during dives. Arterial P_{O_2} also follows a primarily monotonic decline after an initial increase (Ponganis et al., 2009). Similar patterns were observed in the elephant seal and California sea lion, except gas exchange with the lungs ceases at some point during their dives (McDonald and Ponganis, 2012; Meir et al., 2009). Patterns with similar monotonic declines were observed in the turtles, but not consistently (Figs 5 and 6). There were also P_{O_2} profiles that followed an opposite pattern, increasing throughout submergences over 20 min in duration (Fig. 5). No such variation has been observed in arterial P_{O_2} profiles of penguins or pinnipeds.

The observed variation in arterial P_{O_2} profiles reinforces the biological significance of the physiological and anatomical differences between turtles and mammals and birds. Because mammals and birds have separated right and left ventricles in the heart, to the extent gas exchange is maintained during submergences (throughout dives for emperor penguins and for the initial and end portions of dives for pinnipeds), arterial blood gas profiles will closely reflect lung gas tensions (Ponganis et al., 2010, 2009). In contrast, arterial P_{O_2} values in freshwater turtles will also reflect the magnitude and direction of cardiac shunting. Further, as discussed above, turtles, with their low O_2 requirements, high buffering capacity and anoxia tolerance, do not need to maintain high, stable blood O_2 levels.

Prior studies

Our results are also inconsistent with earlier laboratory studies on freshwater turtles using serial sampling or extracorporeal catheter loops (Burggren and Shelton, 1979; Burggren et al., 1989; White et al., 1989). In these studies, arterial P_{O_2} primarily followed a pattern of steady decline over the course of voluntary diving or breath holds (Burggren and Shelton, 1979; Burggren et al., 1989; White et al., 1989). Deviations from this general pattern have been observed, including arterial P_{O_2} oscillations (increases and decreases) within submergences of *T. scripta* (Burggren and

Shelton, 1979). However, in that study, an overall decline in P_{O_2} was observed by the end of submergences (Burggren and Shelton, 1979). In *C. longicollis*, arterial P_{O_2} oscillated up and down, but remained generally constant ('arterial homeostasis') throughout submergences (Burggren et al., 1989). In both studies, these patterns occurred only in a minority of submergences, with most of the submergences following the pattern of steady P_{O_2} decline (Burggren and Shelton, 1979; Burggren et al., 1989). In addition, arterial P_{O_2} values in earlier studies were generally higher than observed in the present study during submergences (Burggren and Shelton, 1979; Burggren et al., 1989; Crossley et al., 1998; Hicks and Wang, 1999; Platzack and Hicks, 2001).

There are several reasons why our results, showing no consistent arterial P_{O_2} pattern and lower arterial P_{O_2} values, may differ from these earlier studies. It is possible that our results differ because the recorder attached to the carapace placed a burden on the turtles that affected O_2 transport in a way that altered arterial O_2 levels. However, we believe this is unlikely because the recorder weighed less than 30 g in water, the behavior of turtles was similar to behavior prior to instrumentation, and turtles can adjust buoyancy to accommodate additional weight (Jackson, 1969). Rather, the level of disturbance to turtles in past experiments may account for the different results.

Past experiments measured blood O_2 levels using serial blood sampling and/or some level of manipulation or impediment to movement, including turtles constrained in small tanks with a small breathing hole (Burggren and Shelton, 1979; Burggren et al., 1989; Glass et al., 1983; Jackson, 1973; White et al., 1989) or turtles tethered to external devices, such as catheters (Burggren and Shelton, 1979; Burggren et al., 1989; Glass et al., 1983; Krosniunas and Hicks, 2003; White et al., 1989). These types of disturbances have been shown to affect the behavior and physiology of reptiles and birds (Gaunt and Gans, 1969; de Villiers et al., 2006; Wright et al., 1992). Similarly, results from early laboratory studies on physiological responses of forcibly submerged marine mammals and birds were not consistently reproduced in freely diving animals (Jobsis et al., 2001). Thus, a primary goal of this study was to understand arterial O_2 levels with minimal disturbance to the turtles. Although a P_{O_2} electrode was implanted and turtles had a recorder on their carapace, the system was self-contained and turtles were otherwise undisturbed. Investigators and others were only in the room for brief periods of time during the 48-h period. Experiments were conducted in the same room and tanks the turtles had acclimated to for the previous 4 weeks. Turtles were able to move freely without restraints and had unfettered access to the surface in these large tanks. Thus, the results in the present study may differ owing to the diminished potential for disturbances confounding physiological responses.

Finally, prior studies, using serial or continuous blood sampling, could not produce the comprehensive P_{O_2} profiles obtained here. We measured P_{O_2} in 529 submergences over a continuous 48-h period in six turtles. The many variations in arterial P_{O_2} profiles observed in the present study included the steady decline, arterial homeostasis and arterial oscillations profiles observed in past studies (Figs 5 and 6). Further, the high sampling rate allowed for a more complete picture of each profile that intermittent blood sampling could easily miss. Thus, with the ability to collect P_{O_2} data at 1 Hz for days, additional P_{O_2} profiles were revealed that were not clearly observed in past studies and the lack of a typical profile was more apparent. Ultimately, this study provides a more complete picture of arterial P_{O_2} profiles than has been observed in past studies.

Conclusions

This first study of arterial P_{O_2} in untethered, undisturbed, freely swimming aquatic turtles has revealed significant differences from past studies of *T. scripta*. Undisturbed, freely diving turtles do not maintain high or stable arterial P_{O_2}, but rather exhibit extreme variation in arterial P_{O_2} across routine activities. Overall, arterial P_{O_2} is also lower during all activities than in previous studies and is not elevated during ventilatory periods. Changes in cardiac shunting patterns likely account for the variation in P_{O_2} profiles and lower values. However, the lack of relationship between P_{O_2} and any activity suggests that cardiac shunts are not regulated to maintain specific arterial P_{O_2} values. Rather, these data support the hypothesis that cardiac shunting is the passive by-product of the factors that alter pulmonary and systemic vascular resistances.

Future work should investigate whether arterial P_{O_2} values are similarly low and variable in other chelonian species, including turtles from different habitats, such as tortoises or marine turtles. Diving marine turtles may have different O_2 transport requirements because lung collapse at depth cuts off gas exchange, making the lung O_2 store inaccessible during deep dives.

Finally, the value of chronically implanted P_{O_2} microelectrodes is also demonstrated in this study. Although arterial P_{O_2} had been studied in freshwater turtles using traditional intermittent blood sampling methods, the patterns and values under different activities were not apparent. Using the implanted P_{O_2} microelectrode and self-contained data logger allowed the turtle to be completely isolated from investigator presence and not tethered to catheters or external equipment. This low level of disturbance allowed us to obtain arterial P_{O_2} measurements in a wide range of activities without being present. The next step will be to examine blood O_2 levels in fully natural conditions by deploying the instruments on freshwater turtles residing in ponds or lakes. The miniaturization of electronics also means investigators can start making these types of measurements on smaller, terrestrial animals. With the use of self-contained physiological loggers, we can finally begin to fully understand the physiological ecology of these animals under natural conditions outside the laboratory.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

C.L.W. and J.W.H. designed and performed the study, C.L.W. analyzed the data, and C.L.W. and J.W.H. prepared the manuscript.

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Supplementary information

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