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RESEARCH ARTICLE

High-altitude diving in river otters: coping with combined hypoxic stresses

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SUMMARY

River otters (*Lontra canadensis*) are highly active, semi-aquatic mammals indigenous to a range of elevations and represent an appropriate model for assessing the physiological responses to diving at altitude. In this study, we performed blood gas analyses and compared blood chemistry of river otters from a high-elevation (2357 m) population at Yellowstone Lake with a sea-level population along the Pacific coast. Comparisons of oxygen dissociation curves (ODC) revealed no significant difference in hemoglobin–oxygen (Hb–O_2) binding affinity between the two populations – potentially because of demands for tissue oxygenation. Instead, high-elevation otters had greater Hb concentrations (18.7 g dl^{-1}) than sea-level otters (15.6 g dl^{-1}). Yellowstone otters displayed higher levels of the vasodilator nitric oxide (NO), and half the concentration of the serum protein albumin, possibly to compensate for increased blood viscosity. Despite compensation in several hematological and serological parameters, theoretical aerobic dive limits (ADL) were similar between high-elevation and sea-level otters because of the lower availability of O_2 at altitude. Our results suggest that recent disruptions to the Yellowstone Lake food web could be detrimental to otters because at this high elevation, constraints on diving may limit their ability to switch to prey in a deep-water environment.

Key words: albumin, chloride shift, hemoglobin binding affinity, invasive species, *Lontra canadensis*, nitric oxide, oxygen dissociation curve.

INTRODUCTION

Two of the most physiologically challenging conditions encountered by air-breathing animals are the lower partial pressure of oxygen (P_{O_2}) at high-altitude and O_2 deprivation during breath-hold diving (Butler and Jones, 1997; Kooyman and Ponganis, 1998; Ramirez et al., 2007). Animals diving at high elevation, however, are faced simultaneously with both of these hypoxic conditions, and their physiological responses to low O_2 availability may be unique. For example, many species native to high elevation have hemoglobin (Hb) with increased O_2-binding affinity, which enhances the uptake of O_2 at the lungs (Lenfant et al., 1971; Storz et al., 2010). Hence, the P_{50} (P_{O_2} at which Hb is 50% saturated with O_2) of these animals is typically lower than that of sea-level residents (León-Velarde et al., 1996a; Storz, 2007). In most mammals, Hb–O_2 affinity is modulated by the allosteric effector 2,3-bisphosphoglycerate (2,3-BPG) (Lenfant et al., 1971), which binds to Hb and promotes O_2 unloading. Hb–O_2 affinity can also be lowered *via* decreases in pH (increased H^+ concentration), increases in carbon dioxide (CO_2) in the blood, increases in temperature, and/or other effectors such as chloride (Cl^-) and nitric oxide (NO) (Mihov et al., 2009; Monge and Léon-Velarde, 1991; Samaja et al., 2003).

Although increased Hb–O_2 affinities have been reported for some diving species as well (MacArthur, 1984; Snyder, 1983), sea-level divers generally encounter well-oxygenated conditions during surface breathing, thereby reducing their need to modulate Hb–O_2 affinity (Kooyman, 1989; Ramirez et al., 2007). Instead, dive duration of these animals often depends on O_2 stores in the lungs, blood and muscles (Kanatous et al., 1999; Kooyman and Ponganis, 1998). Both diving and high-altitude species can enhance O_2 storage *via* an increase in Hb concentration achieved by higher red blood cell (RBC) counts and increased hematocrit (Hct). Indeed, erythrocytosis (elevated RBCs) is a well-known acclimatization response to chronic hypoxia (Hochachka, 1998; Monge and León-Velarde, 1991). However, erythrocytosis can increase blood viscosity, placing additional demands on the heart (Hedrick and Duffield, 1991; Ramirez et al., 2007). Consequently, many shallow-diving species have Hct values similar to those of terrestrial animals (MacArthur, 1984; Nieminen et al., 2007; Weiss et al., 1994), while native high-elevation species typically display low to moderate Hct levels (Monge and León-Velarde, 1991; Ramirez et al., 2007).

The combined constraints of diving and altitude hypoxia may be complicated because hypoxic hyperventilation in air leads to the loss of CO_2 in the blood (alkalosis) while breath-hold diving usually results in CO_2 accumulation (acidosis) (Giardina et al., 2004; Jensen, 2004). Alkalosis decreases the P_{50} of blood, which facilitates O_2 loading from a hypoxic environment but also requires the tissues to function at a lower P_{O_2} to create the necessary diffusion gradient for O_2 transport to the capillaries (Jensen, 2004; Lenfant et al., 1971). A diving animal, however, may reap a benefit at altitude because the accumulation of CO_2 while breath holding increases the P_{50}, thereby improving O_2 delivery to the tissues (Butler and Jones, 1997; Snyder, 1983). The extent to which these effects collectively benefit or limit an animal may depend on the blood's electrochemical environment. For example, the chloride shift (movement of Cl^- from the plasma into the RBCs as blood flows from arterial to venous capillaries) shuttles CO_2 as bicarbonate (HCO_3^-) from the RBCs, thereby enhancing the Hb buffering capacity to accommodate H^+ and unload O_2 (Prange et al., 2001). Animals with a large chloride shift may be able to transport more CO_2 in the blood with a smaller change in the plasma acid–base status (Westen and Prange, 2003).

To investigate O_2 delivery in a diving animal indigenous to a range of elevations we studied river otters, *Lontra canadensis* (Schreber 1777). This species occurs across much of North America and performs foraging dives along coastal shorelines as well as at high-elevation lakes and streams (Melquist et al., 2003). As semi-aquatic piscivores, foraging otters exert themselves during submerged chases and rely on their limbs and tail for propulsion rather than solely on body undulation (Fish, 1994). Thus, muscle O_2 demands can be high for diving otters and could limit the duration and depth of their dives (Davis et al., 2004; Nolet et al., 1993; Pfeiffer and Culik, 1998). Moreover, the cold temperatures of high-latitude marine systems and high-elevation lakes increase an animal's energetic demands (Kruuk et al., 1994; Scholander et al., 1950). Thus, the capacity of river otters to dive at altitudes with low P_{O_2} and cold water temperatures may depend on enhancing Hb delivery of O_2 to tissues.

One of the few native high-altitude populations of river otters occurs at Yellowstone Lake, in Yellowstone National Park. Otters have inhabited the Yellowstone plateau since the end of the last glaciation (~11,000 years), providing ample time and a sufficient number of generations for the population to adapt to high-altitude diving (Frappell et al., 2007). However, for otters in Yellowstone Lake, the challenge of successfully foraging at altitude has been heightened by a recent population decline in the native cutthroat trout, *Oncorhynchus clarki bouvieri* (Koel et al., 2005). Cutthroat trout are important prey for otters in Yellowstone Lake, and during the spawning season provide an accessible and lipid-rich food source (Crait and Ben-David, 2006). For otters in this ecosystem, the only comparable alternative prey is the recently introduced lake trout (*Salvelinus namaycush*). However, lake trout inhabit deeper water (up to 40 m) than cutthroat trout, thereby requiring otters to perform extended and more energetically demanding dives (Crait and Ben-David, 2006).

To elucidate mechanisms that facilitate diving at high altitude, and to assess the ability of Yellowstone otters to switch from feeding on cutthroat trout to feeding on lake trout, we performed blood gas analyses and compared the blood chemistry of animals from Yellowstone Lake to a sea-level population inhabiting a marine ecosystem. We hypothesized that if Yellowstone otters are primarily constrained by altitude hypoxia, they should display increased Hb–O_2 affinity (lower P_{50}), potentially driven by a reduction in 2,3-BPG and/or Cl^- (Samaja et al., 2003; Westen and Prange, 2003). Under this scenario, otters could forage on deep-water prey through enhanced O_2 storage *via* elevated Hb concentration. However, if otters are largely constrained by O_2 delivery to tissues during energetically demanding dives, increased Hb–O_2 affinity may heighten the risk of tissue hypoxia. In this case, we predicted that otters would increase O_2 storage and delivery to tissues *via* elevated Hb concentration (Hammond et al., 2001; Sawin, 1970). Under this second scenario, the limitations of low ambient P_{O_2}, combined with increased blood viscosity incurred by further erythrocytosis, would likely prevent these semi-aquatic mammals from prey shifting to deep-water lake trout.

MATERIALS AND METHODS

Otter trapping

Five river otters (4 males and 1 female, >1 year old) were captured from 4 to 11 June 2005 at Yellowstone Lake, Yellowstone National Park (YNP), WY, USA [elevation 2357 m; 76% of O_2 availability at sea level; barometric pressure 76.34 kPa; study area as described previously (Crait and Ben-David, 2006)]. In addition, 6 river otters (2 males and 3 females, >1 year old; and 1 female <1 year old) were captured from a sea-level population in the San Juan Islands (SJI), WA, USA, between 29 July and 2 August 2005 [barometric pressure 101.88 kPa; study area as described previously (Gaydos et al., 2007)]. Mean body mass was similar for the two sample groups (YNP 8.64 kg; SJI 8.68 kg). Otters were captured with No. 11 Sleepy Creek® leg-hold traps (Sterling Fur and Tool Co., Sterling, OH, USA) monitored by trap transmitters (Telonics, Mesa, AZ, USA). Otters were anesthetized with Telazol (9 mg kg^{-1}; A. H. Robins, Richmond, VA, USA) administered with Telinject® darts (Telinject, Saugus, CA, USA) and a blowgun (Blundell et al., 1999). All methods were approved by an Independent Animal Care and Use Committee at the University of Wyoming.

Blood chemistry and gas analyses

We collected ~20 ml of whole blood from each otter *via* jugular venipuncture, following previously described methods (Bowyer et al., 2003). A 4 ml portion of the sample was preserved in lithium heparin (green top Vacutainer®; Becton Dickinson Labware, Franklin Lakes, NJ, USA) and immediately stored on ice until blood gas analyses were performed within 1 h. All blood gas measurements were performed at 37°C. Approximately 2 ml of the blood sample was equilibrated with simulated arterial and venous gas mixtures generated with a Wösthoff gas mixing pump in a temperature- and humidity-controlled IL 237 tonometer (Instrumentation Laboratories, Lexington, MA, USA) and were adjusted for differences in P_{O_2} and P_{CO_2} between the two elevations. For example, at sea level a simulated arterial gas mixture of 9% O_2, 5% CO_2, with balance N_2, generated P_{O_2}=8.6 kPa and P_{CO_2}=5.3 kPa, while a high-elevation arterial gas mixture of 12% O_2, 7% CO_2, balance N_2, yielded P_{O_2}=8.7 kPa and P_{CO_2}=4.7 kPa. Aliquots of 100–150 µl were drawn from the sample for analysis of pH, P_{O_2}, P_{CO_2}, $[Na^+]$, $[K^+]$ and $[Cl^-]$ in a Radiometer ABL 505 blood gas/electrolyte analyzer and OSM-3 hemoximeter (O_2 saturation, Hb; Radiometer, Copenhagen, Denmark). These instruments are self-calibrating using internal standards.

We used two approaches to compare O_2 binding affinity between high-elevation and sea-level otters. First, we constructed a physiological oxygen dissociation curve (ODC) to simulate *in vivo* changes in Hb–O_2 binding when shifting between arterial and venous blood (Lapennas, 1983; Powell, 2003). The physiological ODC is typically steeper than a standard curve measured at pH 7.4 (Powell, 2003) and was used to approximate conditions faced by otters when delivering O_2 to tissues. In this approach, the percentage of O_2 and CO_2 in the gas mixtures was varied and equilibrated to separate aliquots of blood in order to generate Hb saturation (S_{O_2}) values for the entire curve (e.g. 5% to 95% S_{O_2}). Stepwise equilibrations were performed with a P_{CO_2} of 2.9–8.4 kPa in order to simulate arteriovenous P_{CO_2} conditions typical of the altitudinal range in our study (León-Velarde et al., 1996b; Thews et al., 2004; Virués-Ortega et al., 2004; Wolff, 2008), as well as to investigate O_2 delivery under acidic settings (high P_{CO_2}), such as those found during breath-hold diving, and alkalotic conditions (low P_{CO_2}) resulting from hyperventilation (Giardina et al., 2004; Jensen, 2004). For each P_{CO_2} level, multiple simulated arterial and venous P_{O_2} equilibrations were made, with P_{O_2} ranging from 1.3 to 11.3 kPa. Corresponding mean (±s.e.m.) pH values for these ODCs were 7.25±0.01 for YNP and 7.26±0.01 for SJI. Second, to compare Hb–O_2 binding affinity of river otters with that of species from other studies, we calculated a standard $P_{50\ (7.4)}$ for both study groups. To do so, we generated separate ODCs, and corresponding P_{50} values, at different pH (e.g. P_{50} at pH 7.1, 7.2, 7.3). The CO_2 Bohr coefficient was then derived for both populations from the regression of log P_{50} *vs* pH and used

to correct measured P_{O_2} values to a standard pH 7.4 and 37°C (MacArthur, 1984; Meir et al., 2009; Snyder et al., 1982).

An additional 2 ml of whole blood was preserved in EDTA (purple top Vacutainer®), stored on ice, and transported to the laboratory (within 24 h) for hematological analyses. A 10 ml sample of blood was collected without anticoagulant (red top Vacutainer®) and allowed to clot; serum was separated from cells by centrifugation; 1 ml was stored at –20°C for serum chemistry analyses and the remainder was frozen and stored at –80°C for later NO assays. Blood serum chemistry and hematology analyses were performed for the YNP otters at West Park Hospital in Cody, WY, USA, and for the SJI otters at Phoenix Central Laboratory in Everett, WA, USA. Because samples collected from the SJI otters had to be flown to the mainland, hematological analyses for several individuals were conducted on potentially more degraded samples. Therefore, we chose to report the values generated by the ABL blood gas analyzer and hemoximeter. Hct and RBC values from the ABL were unavailable for some of the SJI otter samples, so we followed methods similar to those suggested by Burness and colleagues for estimating missing values (Burness et al., 2001). We first regressed the laboratory Hb values against those obtained from the ABL (R^2=0.99; P<0.0001), and then used the regression equation and laboratory values to calculate Hct and RBCs. Although using data from either the outside laboratories or the ABL did not affect any subsequent statistical comparisons, the latter measurements yielded more conservative estimates for the hematological differences between YNP and SJI otters, and the values for the latter group are comparable to those reported for coastal river otters in Alaska (Bowyer et al., 2003). All reported serum chemistry data (e.g. strong ions, albumin) for otters from both populations were measured by the commercial labs. Serum bicarbonate values were calculated as HCO_3^-=CO_2×0.95 (Hoover et al., 1984). Finally, data from one individual who suffered from dehydration were excluded from all analyses except to illustrate the relationship between hematological values and NO.

Analyses of 2,3-BPG were performed on the remaining 2 ml of heparinized whole blood. Logistics in the field prevented us from performing these analyses on freshly collected blood, so the samples were frozen within 1 h of collection and stored at –80°C until 2,3-BPG testing. Although freezing whole blood can lead to hemolysis, our 2,3-BPG values are comparable between the YNP and SJI populations because all samples were handled in an identical manner. All laboratory analyses for 2,3-BPG and NO were performed at the University of Wyoming. We precipitated heparinized blood with perchloric acid and used spectrophotometry to measure the concentration of 2,3-BPG with a commercially available test kit (Roche Applied Science, Mannheim, Germany). Serum nitrate (NO_3^-) and nitrite (NO_2^-), the major reaction products of NO (nitrogen oxides, NO_x), were converted to NO with vanadium (III) chloride (VCl_3) in hydrochloric acid (HCl) (Bryan and Grisham, 2007; Ignarro et al., 1993) and then analyzed by a chemiluminescence technique with a Sievers 280i NO Analyzer (General Electric, Boulder, CO, USA).

Statistical analyses

All statistical analyses were conducted with SPSS 18 (SPSS Inc. 2009, www.spss.com). Because of the small sample size, some of the data did not meet the assumptions of normality (Kolmogorov–Smironov test and Q–Q plots) and homogeneity of variance (Levene's test) (Zar, 2009). In these cases we used a Mann–Whitney U-test (Zar, 2009). We used t-tests to compare between groups, including hematology (Hct, Hb and RBCs), concentration of 2,3-BPG, chloride shift and P_{50} values. Based on hematological values for the YNP otters (see Results), we predicted that levels of NO would be higher in that group than in the sea-level population, and used a one-tailed Mann–Whitney U-test to test this prediction. Finally, we explored the relationships between P_{O_2} and Cl^- and HCO_3^- with regression analyses (Zar, 2009). Using the linear regression of Cl^- *vs* P_{O_2}, Cl^- values from simulated arterial blood (P_{O_2}=10.64 kPa) were subtracted from those in the venous gas mixtures (P_{O_2}=4 kPa) to examine the magnitude of the chloride shift (Prange et al., 2001; Westen and Prange, 2003).

RESULTS

River otters from the high altitude, YNP population had significantly higher Hct, total Hb and RBCs than those from the SJI sea-level population (P<0.05; Table 1). Also, YNP otters had significantly lower levels of serum albumin, sodium (Na^+) and Cl^- than otters from the sea-level population (P<0.05; Table 1), and they had significantly higher levels of HCO_3^- and NO_x (P<0.05; Table 1). The level of NO_x in the dehydrated otter from SJI was extremely high (27.2 μmol l^{-1}) and corresponded with similarly high values of Hct (58.3%) and RBCs (15.1×10^6 μl^{-1}). We detected no difference in the mean concentration of 2,3-BPG between the high-elevation group (mean ± s.e.m.: 2.1±0.2 mmol l^{-1}) and sea-level group (2.0±0.5 mmol l^{-1}); however, the absolute measurements of 2,3-BPG should be interpreted with caution because they were performed on thawed blood. Indeed, previous studies reported a higher 2,3-BPG concentration of 3.8 mmol l^{-1} for North American river otters (Bunn et al., 1974). All other comparisons of serum chemistry, including total protein and hematology between high-altitude and sea-level otters were not significant at the α=0.05 level (Table 1).

An overall comparison of O_2 concentration curves for the two groups suggested that at P_{O_2} above 7.7 kPa the high-elevation YNP group had a significantly higher blood O_2 concentration than the sea-level group (P=0.03), coinciding with the aforementioned elevated Hb levels (Table 1, Fig. 1A). A comparison of the ODC

Table 1. Hematology and blood chemistry values for river otters captured at an altitude of 2357 m and at sea level

Variable	Yellowstone	San Juan Islands
Total protein (g dl^{-1})	7.1±0.1	7.0±0.5
Albumin (g dl^{-1})	1.3±0.0	2.9±0.1*
Glucose (mg dl^{-1})	115.8±12.3	127.0±5.4
Sodium (mequiv. l^{-1})	149.6±1.0	156.0±2.4*
Potassium (mequiv. l^{-1})	3.8±0.2	3.9±0.1
Chloride (mequiv. l^{-1})	110.4±1.1	119.0±2.6*
Bicarbonate [HCO_3^-] (mequiv. l^{-1} calculated)	21.1±0.6	18.8±0.6*
Chloride shift (mmol l^{-1})	11.5±3.3	12.5±5.9
[HCO_3^-] arterial–venous difference (mmol l^{-1})	7.0±1.2	3.6±1.6
Calcium (mg dl^{-1})	8.6±0.2	8.4±0.3
Hb (g dl^{-1})	18.7±0.2	15.6±0.2*
RBCs ($\times10^6$ μl^{-1})	10.6±0.3	9.1±0.5*
Hct (%)	51.4±3.6	43.4±3.9*
NO_x (μmol l^{-1})	10.2±1.8	6.5±0.8*

River otters were captured at high altitude (2357 m, Yellowstone Lake, WY, USA) in June 2005 (N=5) and at sea level (San Juan Islands, WA, USA) in August 2005 (N=5).

Values are means ± s.e.m. All blood chemistry data were obtained from serum except chloride shift and [HCO_3^-] arterial–venous difference, which were measured from plasma.

Hb, hemoglobin; Hct, hematocrit.

*Significant difference (P<0.05; Mann–Whitney and t-tests).

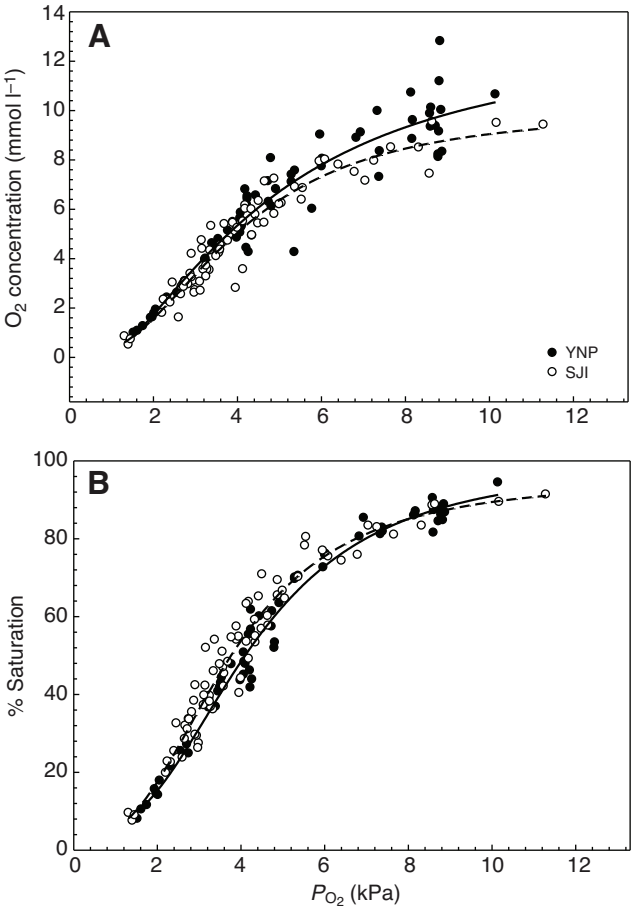

Fig. 1. Oxygen dissociation curves (ODCs) relating O_2 concentration (A) and percentage Hb saturation (B) to P_{O_2} for river otters captured at Yellowstone Lake (YNP, high-altitude population) and the San Juan Islands (SJI, sea-level population), summer 2005. Curves represent physiological conditions and were generated from simulated arterial and venous blood. Mean (±s.e.m.) pH values were 7.25±0.01 for YNP and 7.26±0.01 for SJI otters. See Materials and methods for description.

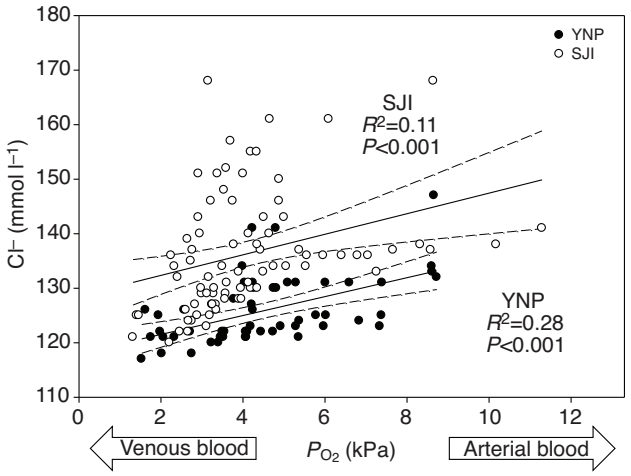

Fig. 2. The 95% confidence intervals for the relationship between chloride and P_{O_2} in venous and arterial blood of river otters. The arteriovenous decrease in Cl^- concentration represents the 'chloride shift' as Cl^- moves from plasma into red blood cells in exchange for bicarbonate. River otters were captured at Yellowstone Lake (YNP, high-altitude population) and the San Juan Islands (SJI, sea-level population), summer 2005. Chloride values generated from simulated arterial and venous blood. Mean (±s.e.m.) pH values were 7.25±0.01 for YNP and 7.26±0.01 for SJI otters. See Materials and methods for description.

and P_{50} values for percentage Hb saturation was not statistically significant between the two groups (P=0.065; Fig. 1B). The CO_2 Bohr effect, $\Delta\log P_{50}/\Delta$pH, was –0.58±0.03 (mean ± s.e.m.) for YNP and –0.44±0.13 for the SJI otters and fell within the range typically reported for diving mammals (Lenfant et al., 1970; Meir and Ponganis, 2009). Mean standard $P_{50(7.4)}$ was similar for the two groups (26.5±0.25 for YNP and 25.6±0.95 for SJI, means ± s.e.m.). The magnitude of the chloride shift and the arterial–venous difference in HCO_3^- were not significantly different between the two populations (P>0.05; Fig. 2, Table 1). Finally, although sex ratios differed between the two sample groups, we found no significant difference (P>0.05) for any blood measurements based on sex.

To assess the capacity of YNP otters to prey switch from native cutthroat trout to non-native lake trout, we used our measurements of Hb concentration to calculate theoretical aerobic dive limits (ADL; defined as the maximum breath-hold possible without an increase in blood lactate concentration during or after a dive) (Kooyman, 1989) for both otter populations, following procedures described previously (Ben-David et al., 2000). It should be noted that a theoretical ADL based on usable O_2 stores is not equivalent to the ADL as originally defined, because an animal usually does not consume all of its O_2 during a single dive, and at times may exceed its theoretical ADL (Butler, 2006) (but see Halsey, 2011). For all calculations we assumed that lung volume and myoglobin (Mb) concentrations of the two populations were identical and that blood volume was 9% of body mass [(Kooyman, 1989) for sea otter (*Enhydra lutris*)] (T. Williams, personal communication); assumptions that may be questioned but cannot be refuted without additional data. Therefore, these calculations were meant to provide an estimate of the impact that changes in Hb and Hct have on theoretical ADL. To calculate theoretical ADL, we divided the total body O_2 store (ml O_2 kg^{-1}) by swimming metabolic rate (Ben-David et al., 2000; Pfeiffer and Culik, 1998). This produced a theoretical ADL of 55.1 s for YNP otters and 53.6 s for the SJI animals.

DISCUSSION

Our comparison of river otters from high-elevation and sea-level populations indicates that these semi-aquatic mammals utilize a suite of physiological mechanisms to overcome the compounded hypoxic conditions encountered when diving at high altitude. Despite diving at an elevation of over 2000 m, YNP otters did not display a significant difference in Hb–O_2 affinity relative to their sea-level counterparts. Instead, to enhance loading of O_2, and to protect the tissues from hypoxia during energetically demanding dives, YNP otters increase Hb concentration. Although this response is typical of a lowland species acclimatizing to the relative hypoxia of altitude, in YNP otters it is also coupled with an increase in NO and a reduction in serum albumin, possibly to mitigate the effects of higher blood viscosity.

Hematology and serum chemistry

Individuals from both populations had hematological values consistent with those of low-elevation river otters; however, YNP otters exhibited Hct and Hb levels at the upper end of published values for this species (e.g. Belfiore, 2008; Davis et al., 1992; Kimber and Kollias, 2005; Reed-Smith, 2008; Serfass et al., 1993; Tocidlowski et al., 2000). Hct levels of YNP (51.4%) and SJI otters

(43.4%) were within the mid- to upper-range of reported measurements for other semi-aquatic mammals [e.g. muskrat *Ondatra zibethicus*, 41.1% (MacArthur, 1984) and star-nosed mole *Condylura cristata*, 50.5% (McIntyre et al., 2002)]. Elevated Hct, RBC and Hb values may indicate a greater diving capacity (Ramirez et al., 2007). For example, semi-aquatic muskrats increase Hct during winter, when performing longer dives beneath ice (MacArthur, 1984). Indeed some of the highest (>60%) Hct levels are found in deep-diving marine mammals (Hedrick and Duffield, 1991; Lenfant et al., 1970; Qvist et al., 1986; Ridgway and Johnston, 1966), many of which regulate circulating levels *via* splenic sequestration and release of erythrocytes (Castellini et al., 2006; Kooyman and Ponganis, 1998). In addition, increased RBC production, mediated by the hormone erythropoietin, is a common acclimatization response to chronic hypoxia (de Bruijn et al., 2008; Hochachka, 1998; Villafuerte et al., 2004). For example, many lowland-adapted humans experience erythrocytosis when visiting high elevations (Beall, 2006; Monge and León-Velarde, 1991); and harbor seals (*Phoca vitulina*), a species with intrinsically large O_2 stores, further increase Hct when exposed to altitude (Kodama et al., 1977).

Although erythrocytosis increases maximal O_2 concentration in the blood, higher Hct may not lead to full O_2 saturation because of low ambient P_{O_2} at altitude. High-elevation animals may, however, be able to deliver as much O_2 to the tissues as their sea-level counterparts because O_2 transport occurs on the steep portion of the ODC where a smaller decrease in tissue P_{O_2} is required to unload O_2 (Monge and León-Velarde, 1991; Storz, 2007) (Fig. 1A). Thus, the greater Hb concentration found in YNP otters both increases O_2 carrying capacity and enhances O_2 delivery to the tissues. For a high-intensity swimming mammal, this set of responses could serve to protect the tissues from hypoxia.

Despite the advantages for O_2 storage, elevated Hb and Hct can increase blood viscosity, raising blood pressure, increasing the risk of clotting and potentially compromising O_2 transport (Hedrick and Duffield, 1991; Ramirez et al., 2007). Indeed, elevated Hct and Hb are uncommon in species genetically adapted to hypoxia (e.g. Monge and León-Velarde, 1991; Ramirez et al., 2007). Given that Hct levels in the YNP otters were among the highest reported for the species, these animals may be susceptible to reduced blood flow. Furthermore, increased blood viscosity can constrain maximal swimming speeds (Hedrick and Duffield, 1991), a crucial aspect of foraging success for these active divers.

The higher levels of serum NO in YNP otters may help to offset the hypertensive effects of increased blood viscosity. NO is a strong vasodilator, which diffuses from the endothelium and acts on soluble guanylyl cyclase in the vascular smooth muscle of blood vessels (Fleming and Busse, 2003; Ruschitzka et al., 2000). Endothelial NO production is stimulated by increases in viscosity-induced shear stress on vascular tissues (Furchgott and Vanhoutte, 1989; Martini et al., 2005). Consequently, moderate increases in Hct can lower blood pressure *via* NO-mediated vasodilatation (Martini et al., 2005; Salazar Vázquez et al., 2010; Wilcox et al., 1993). The suggestion that otters may enhance NO production in response to increased blood viscosity is supported by the observation that the one dehydrated SJI individual showed exceedingly high levels of NO, coinciding with an elevated concentration of RBCs and raised Hct. However, similar to findings by Beall and colleagues for Tibetan and Andean highlanders (Beall et al., 2001), we found little correlation between NO and Hct and Hb, suggesting that increased NO synthesis is likely not governed solely by erythrocytosis. Under hypoxic conditions, enhanced NO synthesis may improve blood flow and tissue oxygenation (Ahsan et al., 2005; Bigham et al., 2010; Vogel et al., 2003; Xing et al., 2008). For example, in Tibetans, elevated levels of NO in the lungs and systemic circulation are associated with higher blood flow than found in sea-level humans (Beall et al., 2001; Erzurum et al., 2007). Given the increasing evidence of the importance of NO in regulating blood flow (Jensen, 2009; Singel and Stamler, 2004), its role in counteracting blood viscosity and balancing respiratory tradeoffs in high-altitude divers warrants further investigation.

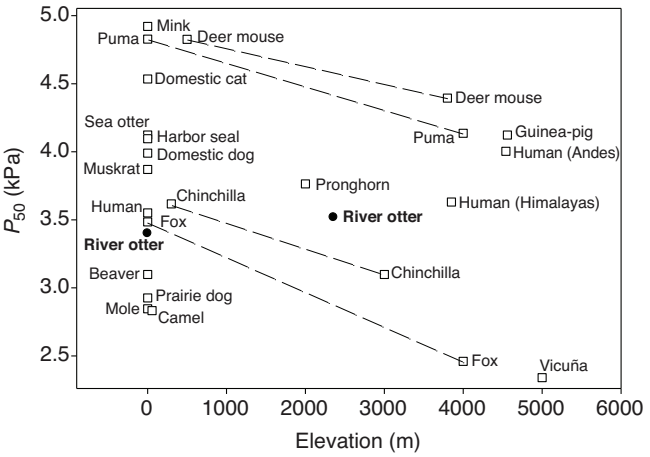

Fig. 3. Mean P_{50} values for several mammalian species across a range of elevations. Data from: beaver (*Castor fiber*) (Clausen and Ersland, 1968), chinchilla (*Chinchilla* sp.) (Ostojic et al., 2002), deer mouse (*Peromyscus* sp.) (Snyder et al., 1982), domestic cat (*Felis catus*) and dog (*Canis familiaris*) (Cambier et al., 2004), Andean and sea-level human (Winslow et al., 1981), Himalayan human (Samaja et al., 1979), fox (*Dusicyon* sp.) and puma (*Puma concolor*) (León-Velarde et al., 1996a), guinea-pig (*Cavia* sp.) (Turek et al., 1980), muskrat (MacArthur, 1984), mink (Scott et al., 1939), mole (*Talpa europaea*) (Jelkmann et al., 1981), prairie dog (*Cynomys ludovicianus*) (Hall, 1965), pronghorn (*Antilocapra americana*) (Dhindsa et al., 1974), sea otter and harbor seal (Lenfant et al., 1970), camel (*Camelus dromedarius*) and vicuña (*Vicugna vicugna*) (Jürgens et al., 1988). Lines are shown for studies in which different populations were compared across elevations. pH 7.4 and 37°C, except guinea-pig (37.5°C) and pronghorn (38°C).

The elevated NO measurements in YNP otters may also be partly tied to their strikingly low levels of albumin, because hypoalbuminemia can accelerate endothelial production of NO (Bevers et al., 2006). Total serum albumin levels for the YNP population were less than half of the ~3.0 g dl^{-1} found in the SJI animals, as well as those reported in previous otter studies at low elevation (e.g. Reed-Smith, 2008; Tocidlowski et al., 2000). Albumin is a key plasma protein responsible for the maintenance of oncotic pressure, blood buffering and transportation of low solubility metabolites (Baker, 2002; Johnson, 2003), and its levels seldom vary. In mammals, hypoalbuminemia may be associated with protein malnutrition, metabolic acidosis, acute inflammation and impaired liver function (Ballmer, 2001). At high altitude, hypoxia-induced limitations on liver protein synthesis (Imoberdorf et al., 2001; Preedy et al., 1985) and increased urinary protein excretion *via* elevated capillary permeability can decrease albumin levels (Jefferson et al., 2002; Rennie et al., 1971). Nonetheless, consistent with our findings, some apparently healthy human populations native to high altitude exhibit depressed levels of serum albumin [up to 26% lower: 3.1–3.3 compared with 4.2–4.5 g dl^{-1} (Kametas et al., 2004; Shivastava and Malhotra, 1974)], suggesting that they have developed compensatory mechanisms in other organ systems.

It is possible that reduced serum albumin in the YNP otters, in conjunction with elevated NO, helps to mitigate increases in blood viscosity and improve blood flow. For example, Kametas and colleagues found that Peruvian women native to high elevation had higher Hct, total protein and fibrinogen, but lower albumin concentrations than their lowland counterparts (Kametas et al., 2004). The authors suggested that given higher total protein concentrations at altitude, albumin reductions may offset further increases in plasma viscosity (Kametas et al., 2004). Although albumin reductions may benefit otters at altitude, the cost of limited transport and buffer capacities associated with a greater than 50% reduction in this important blood protein are unknown and merit further study.

ODCs and Hb–O_2 affinity

Our comparisons of ODCs suggest that P_{50} values of YNP otters are similar to those of the sea-level group (Fig. 1B). This may imply that, at 2357 m, the Yellowstone Lake ecosystem presents only a moderate hypoxic stress to river otters that can be addressed with hematological adjustments rather than an increase in Hb–O_2 affinity. Several authors contend that a reduced Hb–O_2 affinity is preferable to promote O_2 delivery to tissues under moderate altitude hypoxia [e.g. up to 5500 m for humans (Samaja et al., 2003) and 6400 m for llamas, *Lama glama* (Banchero and Grover, 1972)]. Furthermore, Villafuerte and colleagues suggested that at mid-level elevations (up to 3800 m), minimal declines in alveolar P_{O2} are sufficient to cause an increase in Hb for maintaining tissue oxygenation in humans (Villafuerte et al., 2004). Although river otters inhabit higher elevations in the Rocky Mountains, they are rarely found in deep-water lakes at these altitudes and instead forage in shallow headwater streams. Thus, investigating Hb–O_2 affinity in relation to diving in otters at more extreme elevations is unlikely.

Alternatively, demands for tissue oxygenation during activity may prevent increased Hb–O_2 affinity in YNP otters, yielding a similar ODC for the two populations. Indeed, the similar P_{50} values in our otter populations is consistent with studies of burrowing mammals that encounter compounded hypoxic conditions due to low O_2 tensions below ground (Lechner, 1976; MacArthur, 1984). For example, Broekman and colleagues found that burrowing naked mole rats (*Cryptomys hottentotus*) had similar P_{50} values across a range of elevations, and suggested that these values were already maximized for aerobic tissue metabolism during below-ground hypoxia (Broekman et al., 2006). Although river otters perform relatively brief and shallow dives that typically last less than 30 s (Ben-David et al., 2000), their foraging bouts often involve high-intensity chases necessary to capture fish. This is especially true for YNP otters that feed on fast salmonid prey. Maintaining a Hb–O_2 affinity similar to that of their lowland counterparts may prevent tissue hypoxia in this population (Meir et al., 2009). Moreover, the moderately higher CO_2 Bohr factor of YNP otters could further enhance O_2 unloading to the tissues (Meir and Ponganis, 2009). Indeed, while the P_{50} of river otters falls within the range of other diving and burrowing species at sea level, demands for tissue oxygenation associated with diving and fish predation may explain why YNP otters did not show an altitudinal decrease in P_{50} often found in other species at high elevation (Fig. 3).

Consistent with their corresponding P_{50} values, the arteriovenous chloride shift was similar in magnitude between the two otter groups (Fig. 2, Table 1). A large chloride shift may indicate more Cl^- binding to Hb to facilitate O_2 unloading (Brix et al., 1990; Westen and Prange, 2003). Although we did not detect a difference in the chloride shift between YNP and SJI otters, our measurements for river otters are higher than reported for some other mammals, including humans (~3 mmol l^{-1}) and cattle [1.7 mmol l^{-1}, *Bos* sp. (Westen and Prange, 2003)]. We suggest two possibilities: first, in addition to 2,3-BPG, Cl^- may be an important modulator of Hb–O_2 binding affinity in river otters. For example, some species that use Cl^- as their primary allosteric effector of Hb display a similarly large chloride shift [e.g. 20–30 mmol l^{-1} for brown bear, *Ursus arctos* (Brix et al., 1990)]. Second, for a semi-aquatic species such as the otter, a large chloride shift may facilitate higher HCO_3^- plasma concentrations and mitigate acidosis-induced pH changes during breath-hold dives (Prange et al., 2001). It is unclear why the chloride shift was similar between the populations, given the higher Hct in YNP otters (Westen and Prange, 2003). We speculate that other cofactors, such as NO, could have competed with Cl^- for binding sites on Hb (Mihov et al., 2009); however, this hypothesis requires further study.

Despite the similarity in chloride shift in the two groups, absolute serum salt concentrations were greater for the SJI otters than for the YNP animals. Though values for both populations fall within previously recorded measurements for river otters (Davis et al., 1992; Tocidlowski et al., 2000), Na^+ and Cl^- concentrations were higher in the SJI otters, possibly as a result of their transitioning between freshwater and marine habitats. For example, freshwater manatees (*Trichechus manatus*) acutely exposed to saltwater showed significant increases in plasma Na^+ and Cl^- (Ortiz et al., 1998). Thus, it is unlikely that the lower serum salt concentrations in YNP otters are related to modulation of Hb–O_2 binding affinity.

CONCLUSION

River otters diving in Yellowstone Lake appear to respond to the chronic hypoxia of altitude primarily via increased Hb concentrations. The accompanied responses of increased NO, reduced albumin, and relatively large chloride and Bohr shifts indicate that these otters are largely constrained by O_2 tissue demands during high-energy dives, favoring increased O_2 storage rather than higher Hb–O_2 affinity. In an ecological context, these results suggest that recent declines in the native cutthroat trout population could be detrimental to river otters in Yellowstone Lake. YNP otters have relatively high-capture success rates when preying on cutthroat trout [e.g. 38–40% per dive (Varley, 1998)]. However, most foraging dives occur in shallow water. YNP otters are unlikely to further modify blood hematology to accommodate the longer and deeper dives required for preying on non-native lake trout. In addition, our calculations of theoretical ADL imply that, given the lower P_{O2} at Yellowstone Lake, a 22% increase in Hct measured in YNP otters provides a mere 1.5 s increase in ADL. Although we did not account for possible changes in Mb concentration in these animals, our findings suggest that compensation in several hematological and serological parameters may only allow YNP otters to keep pace with the diving capacity of their sea-level counterparts. River otters rarely exceed their ADL in a single dive; however, successful foraging involves multiple breath-hold dives where O_2 stores are replenished during short bouts at the surface. It was previously found (Ben-David et al., 2000) that declines in Hb in river otters did not change the duration of an individual dive but rather reduced total submergence time in a foraging bout, thus leading to lower capture success rate. Because diving may limit alterations to Hb–O_2 affinity, and because additional increases in Hb concentration could further increase blood viscosity, it appears unlikely that river otters in Yellowstone Lake have the physiological capacity to successfully prey switch to deep-water lake trout, potentially threatening the persistence of this population.

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