

RESEARCH ARTICLE

Regional variability in diving physiology and behavior in a widely distributed air-breathing marine predator, the South American sea lion (*Otaria byronia*)

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ABSTRACT

Our understanding of how air-breathing marine predators cope with environmental variability is limited by our inadequate knowledge of their ecological and physiological parameters. Because of their wide distribution along both coasts of the sub-continent, South American sea lions (*Otaria byronia*) provide a valuable opportunity to study the behavioral and physiological plasticity of a marine predator in different environments. We measured the oxygen stores and diving behavior of South American sea lions throughout most of its range, allowing us to demonstrate that diving ability and behavior vary across its range. We found no significant differences in mass-specific blood volumes of sea lions among field sites and a negative relationship between mass-specific oxygen storage and size, which suggests that exposure to different habitats and geographical locations better explains oxygen storage capacities and diving capability in South American sea lions than body size alone. The largest animals in our study (individuals from Uruguay) were the shallowest and shortest duration divers, and had the lowest mass-specific total body oxygen stores, while the deepest and longest duration divers (individuals from southern Chile) had significantly larger mass-specific oxygen stores, despite being much smaller animals. Our study suggests that the physiology of air-breathing diving predators is not fixed, but that it can be adjusted, to a certain extent, depending on the ecological setting and or habitat. These adjustments can be thought of as a ‘training effect’: as the animal continues to push its physiological capacity through greater hypoxic exposure, its breath-holding capacity increases.

KEY WORDS: Aerobic dive limit, Diving, Oxygen stores, Pinniped, South America

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INTRODUCTION

Many marine top predators have undergone declines in their abundance, mostly as a consequence of human exploitation (Casini et al., 2009; Heithaus et al., 2008). However, their ability to recover after cessation of exploitation varies widely, and the reasons for such disparities in their recovery are poorly understood, often because of inadequate knowledge of their ecology, physiology and interactions with human activities (Lotze et al., 2011). Air-breathing marine vertebrates face the challenge of having to partition their access to two of the most fundamental resources they require for their existence: breathing air at the surface, and searching and catching prey at depth. A number of physiological adaptations have been key in their evolutionary success, particularly their ability to carry sufficient oxygen to fulfill their metabolic demands while exercising underwater (Boyd, 1997; Davis et al., 2004; Kooyman, 1989; Kooyman et al., 1983, 1980; McDonald and Ponganis, 2014; Ponganis et al., 1992; Villegas-Amtmann and Costa, 2010). Consequently, the estimation of the total body oxygen stored in the lungs, blood and muscle can provide insight into how these species operate in their respective environments (Costa, 2007; Kooyman, 1989; Kooyman and Ponganis, 1998). As the estimation of total body oxygen stores requires sampling and handling of animals, our knowledge is biased towards amphibious species that can be handled on land (pinnipeds, penguins and reptiles), or small cetaceans and sirenians that can be sampled in captivity or post mortem (Noren et al., 2002; Noren and Williams, 2000). While the differences in oxygen stores and diving physiology caused by intrinsic factors, such as age, size and gender have been examined (Fowler et al., 2007; Hassrick et al., 2010; MacArthur et al., 2001; Ponganis et al., 1999; Richmond et al., 2006; Weise and Costa, 2007), the role of the extrinsic environmental changes on diving physiology has only recently been considered (Costa et al., 2004; Maekawa and Kato, 2015; Thometz et al., 2015; Villegas-Amtmann et al., 2012).

Given the logistical difficulties in working with free-ranging marine birds, mammals or reptiles, comparisons of the diving behavior and physiology for the same species in different habitats has been limited. Previous studies have focused on particular colonies of land (or ice) breeders [e.g. emperor penguins, *Aptenodytes forsteri*; rhinoceros auklets, *Cerorhinca monocerata*; Weddell seals, *Leptonychotes weddelli*; northern elephant seals, *Mirounga angustirostris*; etc. (Burns, 1999; Hassrick et al., 2013; Meir et al., 2013; Ponganis et al., 2011; Yamamoto et al., 2011)], or on the diving behavior of specific species with restricted distributions [e.g. Australian sea lion, *Neophoca cinerea* (Fowler et al., 2007); New Zealand sea lion, *Phocarctos hookeri* (Costa et al., 1998; Leung et al., 2014); Galapagos sea lion, *Zalophus wollabaecki* (Villegas-Amtmann and Costa, 2010)].

The South American sea lion, *Otaria byronia* (Blainville), is an abundant sea lion species (>400,000 individuals) that ranges along both coasts of South America (Crespo et al., 2012), covering a coastline of over 100,000 km (including inner channel waters, islands and fjords) (Fig. 1). Throughout its distribution, this sea lion occupies an ample variety of coastal habitats, from exposed coastline and narrow continental shelf to protected inland waters with a wide continental shelf. This makes the South American sea lion a particularly good model system with which to investigate how an air-breathing marine vertebrate might display plasticity in both its behavioral and physiological traits in response to quite different environmental conditions. Although a few studies have examined the diving behavior of South American sea lions, they have been limited to its Atlantic range, from Uruguay (Riet-Sapirza et al., 2013; Rodriguez et al., 2013) to Argentinean Patagonia (Campagna et al., 2001; Werner and Campagna, 1995) and the Falkland/Malvinas Islands (Baylis et al., 2015a; Thompson et al., 1998). Using time-depth recorders and satellite tags, these studies have found that sea lions are typically benthic foragers restricted to continental shelf waters (Campagna et al., 1995, 2001; Riet-Sapirza et al., 2013; Rodriguez et al., 2013; Thompson et al., 1998; Werner and Campagna, 1995). However, a recent study along the Pacific coast of South America showed that juvenile male sea lions forage pelagically and are not limited to the continental shelf (Hückstädt et al., 2014). Finally, the South American sea lion is the only member of the family Otariidae for which body oxygen stores have not been measured. This combination of different foraging behaviors and a lack of information on body oxygen stores makes the South American sea lion a particularly interesting subject to investigate how different habitats affect diving behavior and

whether physiological capacities vary between different habitats within the same species.

The aerobic dive limit (ADL) is defined as the maximum dive duration before blood lactic acid levels rise as a result of an increase in anaerobic metabolism, and is therefore a function of both oxygen stores and oxygen consumption (metabolic rate) (Costa et al., 2001; Kooyman, 1989; Kooyman et al., 1983, 1980). Therefore, the ADL can be used to determine whether and when sea lions are performing dives that approach their maximum aerobic capacity, which has been reported for other benthic and deep-diving otariids (Costa et al., 2001, 2004). At a population level, such a diving strategy could put a species at risk if they are unable to cope with environmental variability and fluctuations (Boyd, 1997; Costa et al., 2001; Thometz et al., 2015). Although the South American sea lion is categorized as a species of Least Concern (IUCN), the recovery of local stocks in the decades following their commercial harvest differs greatly between regions. The Atlantic sector, where harvesting reduced the sea lion population to less than 10% of its original size, has been only slowly recovering over the last two decades. This contrasts with the rapid recovery observed for the population in the Pacific, even after being affected by the strong 1997/1998 El Niño event (Crespo et al., 2012).

We conducted a comparative study on the diving behavior and physiology of the South American sea lion to determine whether a species occupying different environments exhibits different physiological capacities in response to these different habitats. We hypothesized that South American sea lions would exhibit different diving behaviors as well as different physiological capacities across diverse habitats throughout their range. If present, these differences should allow us to test whether the physiology or the environment (or both) are driving these differences in behavior. We examined these hypotheses by (1) measuring the total body oxygen stores of South American sea lions throughout their entire range, and (2) identifying the differences in their diving behavior.

MATERIALS AND METHODS

Ethics statement

This research was conducted under permits by Subsecretaria de Pesca de Chile (no. 2799/2008, 2396/2009, 1737/2011 and 786/2011), Falkland Islands Government (R14/2001, R14/2014) and DINARA/Ministry of Livestock, Agriculture and Fisheries, Uruguay (572/208). All animal procedures were authorized under the University of California Santa Cruz Institutional Animal Care and Use Committee (IACUC).

We captured South American sea lions ($N=43$) in five different locations throughout the range of the species along the coasts of both the Pacific and Atlantic Oceans (Fig. 1, Table S1). Because of the differences in accessibility and behavior of the animals, the logistics varied between locations. When possible, animals were captured from colonies selecting animals based on healthy appearance and accessibility, by using a custom tapered hoop net with a hole at the end to facilitate animal breathing (northern Chile and Uruguay), or by darting animals using Telazol (2.8 mg kg^{-1}), administered using a 1.5 cc Pnue-Darts syringe and a CO_2 powered tranquilizer gun (Dan-Inject JM Standard, Børkop, Denmark) (Falkland Islands; Baylis et al., 2015a). Animals in central and southern Chile were captured in the water taking advantage of the operation of small purse-seiners, using the method described by Hückstädt et al. (2014). After safe capture (or immobilization in the case of the Falkland Islands), animals were anesthetized using isoflurane gas via a portable vaporizer (Gales and Mattlin, 1998).

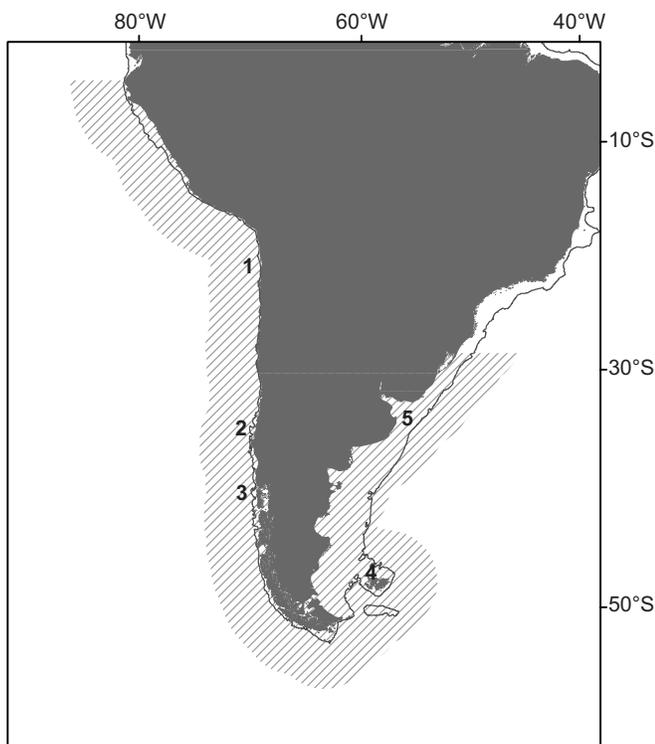


Fig. 1. Distribution of *Otaria byronia* along the Pacific and Atlantic coasts of South America (dashed area). Numbers indicate the location of field sites where animals were captured for this study: (1) northern Chile, (2) central Chile, (3) southern Chile, (4) the Falkland/Malvinas Islands and (5) Uruguay. The solid line represents the continental shelf break (200 m isobath).

Once the animals were anesthetized, we collected morphometric data and blood and muscle samples, instrumented them and, when possible, weighed them before releasing them.

Sample collection and analysis

To estimate individual hematocrit (Hct) on all animals, an initial blood sample was collected from the caudal-gluteal vein into a heparinized Vacutainer. For animals that underwent gas anesthesia, the sample was collected prior to induction, using manual restraint to avoid the decrease in Hct associated with anesthesia (Costa et al., 1998; Ponganis et al., 1992). Hct was measured in triplicate using a micro hematocrit centrifuge. For analysis of total hemoglobin concentration (Hb), two 10 μ l aliquots of the initial heparinized whole blood sample were added to 2.5 ml Drabkin's reagent (Ricca Chemical Co., Arlington, TX, USA) and stored at room temperature in the dark until analysis could be performed. Hb concentration was determined following the methanocyanide hemoglobin photometric method. Once in the laboratory, samples were read at 540 nm in a microplate spectrophotometer (Epoch, BioTek, Winooski, VT, USA), and Hb was calculated using linear regression based on a calibration curve obtained for a series of standards of known Hb concentration (Stanbio Laboratory, Boerne, TX, USA). Mean corpuscular hemoglobin content (MCHC) was calculated as follows:

$$\text{MCHC} = \frac{\text{Hb}}{\text{Hct}} \times 100. \quad (1)$$

We estimated sea lion blood volume using the Evans Blue dilution method (El-Sayed et al., 1995; Swan and Nelson, 1971). After an initial blood sample was collected into a heparinized Vacutainer tube, each individual was injected with Evans Blue dye solution (30 g l⁻¹) at a dose of 0.6 mg kg⁻¹. Syringes were weighed to measure the mass of the injected dye contained in the syringe, and after the dye was injected, blood was drawn into the syringe and flushed at least three times to deliver any remaining dye into the vein. Post-injection blood samples were drawn at 10, 20 and 30 min after the initial dye injection to allow equilibration. All blood samples were then centrifuged shortly after collection, and blood plasma was stored at -20°C until laboratory analysis was conducted, except for the Falkland Islands samples, which were kept cool until they could be frozen within a week of sampling.

A standard curve for estimating blood volume was produced using a series of serially diluted standards of known Evans Blue dye concentration, using the same Evan's Blue dye solution from the fieldwork (El-Sayed et al., 1995; Foldager and Blomqvist, 1991; Swan and Nelson, 1971). Plasma samples were thawed, vortexed and read at 624 and 740 nm in a microplate spectrophotometer (Epoch, BioTek), along with the standard curve samples.

When possible, a muscle biopsy was collected from the dorsal triceps complex during anesthesia. After sterilizing the site with betadine, a ca. 2 cm incision was made in the skin and outer blubber with a scalpel and a 6 mm biopsy punch was used to collect a muscle sample of approximately 50 mg. Samples were frozen at -80°C until analyses. Myoglobin (Mb) was determined following Reynafarje (1963).

Animal instrumentation

South American sea lions were instrumented during handling to measure at-sea location and diving behavior. Animals from northern

(*n*=9) and southern Chile (*n*=10) were instrumented with Sea Mammal Research Unit Fastloc GPS tags (SMRU-GPS, St Andrews University, Scotland) (Sepúlveda et al., 2015); sea lions from central Chile (*n*=4) were instrumented with SPLASH tags (Wildlife Computers, Redmond, WA, USA) (Hückstädt et al., 2014). On the Falkland Islands, animals (*n*=10) were instrumented with MK9 time-depth recorders (Wildlife Computers) and Sirtrack Fastloc-GPS tags (Fastloc I, New Zealand) (Baylis et al., 2015a). In Uruguay, female sea lions (*n*=9) were instrumented with MK9 time-depth recorders (Wildlife Computers) and either a Fastloc-GPS tag (Sirtrack, New Zealand) or an Argos SPOT satellite tag (Wildlife Computers) (Riet-Sapirza et al., 2013). Specifics about instruments programming and resolution are provided in Table S2.

Diving data obtained from the SMRU-GPS tags were processed (zero-offset correction) and summarized on board and transmitted via the ARGOS satellite system (Fedak et al., 2001). Although the dataset is partial, it does include dive depth (m) and dive duration (s). Therefore, for the purposes of this comparative study, we focused on maximum depth of each dive and duration. Archived diving data obtained from other animals (Wildlife Computers MK9), were processed in the laboratory using a custom-written algorithm (IKNOS, Y. Tremblay, Institut de Recherche pour le Développement, France) that performs the zero-offset correction and estimates a suite of parameters from the full resolution diving data.

ARGOS-derived tracks were filtered using a state-space model (Jonsen et al., 2005; Patterson et al., 2008), whereas GPS-derived tracking data were filtered using a speed threshold to remove unrealistic locations (McConnell et al., 1992). Data on location of individual sea lions are presented elsewhere (Hückstädt et al., 2014; Riet-Sapirza et al., 2013). The processed tracking data were then used to linearly interpolate the location for each dive and thus extract the bathymetric depth based on the ETOPO 1-min bathymetric dataset. With these data, we calculated a dive index (diving depth/bathymetric depth), which indicates what portion of the water column is used by the sea lions (Hückstädt et al., 2014).

Data analyses

Unfortunately, we were not able to measure body mass for animals on the Falkland Islands. However, we recorded standard length for all sea lions included in our study, which allowed us to model the relationship between standard length and body mass, and subsequently estimate the mass of individuals for whom we lacked those data. We analyzed the log relationship between body mass (M_b) and standard length (L_s) (*n*=24) as follows (see Fig. S1):

$$\log(M_b) = \beta_0 + \beta_1 \log(L_s) + \varepsilon, \quad (2)$$

where $\varepsilon \sim N(0, \sigma^2)$. To account for uncertainty in the relationship, we used a Bayesian analysis. Priors on parameters β_0 and β_1 were uniformly distributed, and the prior for σ^2 was uniformly greater than zero on $1/\sigma^2$. Posterior distributions and joint posterior samples of all parameters were determined using the program MTG (Metropolis within Gibbs) developed by D. Goodman of Montana State University (Schwarz, 2008). Standard practices (multiple independent chains with low lag-1 autocorrelation) ensured mixing, convergence and stationarity in posterior samples (Cowles and Carlin, 1995; Heidelberger and Welch, 1983; Plummer et al., 2006). Measured standard lengths were then combined with posterior samples of β_0 , β_1 and σ^2 in the above equation to produce a distribution of mass.

We were not able to obtain muscle samples from all sampled individuals, and we were not able to sample muscle tissue for the Falkland Islands animals. However, raw and mass-specific muscle Mb concentration did not significantly differ between sexes (Kruskal–Wallis, $H=1.105$, $P=0.293$) or between field sites (ANOVA, $F_{2,16}=1.465$, $P=0.261$). We therefore assumed that animals from the Falklands had muscle Mb concentrations similar to those of individuals from other field sites. Given the objectives of this work, we estimated Mb values for all animals for which muscle samples were not collected using Monte Carlo simulations. We reconstructed a log-normal distribution of mass-specific Mb values based on all other individuals for which muscle was collected ($n=20$) and calculated mean individual values based on 10,000 random selected samples drawn from the described distribution.

Individual total body oxygen stores (C_{TO_2}) were calculated as follows:

$$C_{TO_2} = C_{aO_2} + C_{vO_2} + C_{MO_2} + C_{LO_2}, \quad (3)$$

where C_{aO_2} is the arterial blood oxygen, C_{vO_2} is the venous blood oxygen, C_{MO_2} is the muscle oxygen and C_{LO_2} is the oxygen in the lungs (Kooyman, 1989; Kooyman and Ponganis, 1998; Villegas-Amtmann and Costa, 2010; Weise and Costa, 2007).

Arterial and venous blood oxygen stores were calculated as follows:

$$C_{aO_2} = V_B \times 0.33B_{BO_2} \times 0.8, \quad (4)$$

$$C_{vO_2} = V_B \times 0.67B_{BO_2} \times S_{VO_2}, \quad (5)$$

where V_B is the blood volume (ml), 0.33 is the fraction of blood in the arteries, 0.66 is the fraction of blood in the veins, B_{BO_2} is the capacitance coefficient of blood ($\text{g Hb l}^{-1} 1.34 \text{ ml O}_2 \text{ g}^{-1} \text{ Hb}$) and S_{VO_2} is the oxygen saturation of venous blood (5% less oxygen than arterial blood, which is assumed to drop from 100% saturation at the beginning of the dive to 20% at the end of the dive) (Kooyman et al., 1980; Ponganis et al., 1992).

Muscle oxygen stores (C_{MO_2}) were calculated as:

$$C_{MO_2} = M_b \times 0.37 \times Mb \times 1.34, \quad (6)$$

where 0.37 corresponds to the fraction of muscle mass in the body, Mb is myoglobin concentration and $1.34 \text{ O}_2 \text{ g}^{-1}$ is the oxygen binding capacity of myoglobin (Kooyman, 1989).

Individual lung oxygen stores (C_{LO_2}) were calculated as follows:

$$C_{LO_2} = V_L \times M_b \times 0.15F_{O_2}, \quad (7)$$

where V_L corresponds to diving lung volume ($V_L=0.5 \times 0.10 \times M_b^{0.95}$) and $0.15F_{O_2}$ is the oxygen that is extracted from air in the lungs (Kooyman, 1989, 1973).

We estimated the calculated aerobic dive limit (cADL) of South American sea lions as: $\text{cADL (min)} = \text{available O}_2 \text{ stores (ml O}_2) / \text{diving metabolic rate (ml O}_2 \text{ min}^{-1})$.

To our knowledge, field metabolic rate (FMR) has not been measured for South American sea lions. However, Dassis et al. (2012) measured submerged swimming metabolic rate in two juvenile individuals held in captivity in Argentina, which can be used as an approximation of the species' FMR. Thus, in order to incorporate uncertainty in our estimation of cADL, we used Monte Carlo simulations by randomly selecting a sample ($n=10,000$) from the reconstructed log-normal distribution of swimming \dot{V}_{O_2} , because \dot{V}_{O_2} cannot be less than zero, as reported by Dassis et al. (2012).

Differences in blood oxygen store parameters between field sites and sexes were analyzed using ANOVA when data were normally distributed, or the Kruskal–Wallis test if the data did not meet the criteria of normality. Linear regression analysis was used to evaluate the relationship between oxygen stores and body mass, as well as the relationship between oxygen store parameters (Hct, Hb, Mb) and diving behavior (dive depth and duration).

RESULTS

We collected physiological (partial or complete) and behavioral data from a total of 43 adult female and juvenile male South American sea lions at five different locations throughout their range between 2009 and 2014. Because of the opportunistic nature of our sampling protocol, particularly at the three field sites in Chile (see Hückstädt et al., 2014), we did not focus on a particular sex or age class.

Standard lengths ranged between 1.46 and 2.0 m for females, and between 1.28 and 1.57 m for juvenile males (Table S1). Individual body masses (measured or estimated) ranged between 68.2 and 160.2 kg for females, and between 52.8 and 116.2 kg for juvenile males (Table 1, Table S1). Body mass varied significantly across sites (ANOVA, $F_{4,42}=5.164$, $P=0.002$); animals from Uruguay were generally larger compared with animals from northern and central Chile (*post hoc* Bonferroni test).

Blood oxygen stores

South American sea lion mean Hct was $46.9 \pm 4.9\%$ ($n=41$) and Hct values were negatively related to individual body mass ($R^2=0.235$, $P=0.001$; Fig. 2A). Juvenile males had significantly higher Hct values than females ($\text{Hct}_{\text{juveniles}}=51.0 \pm 3.9\%$; $\text{Hct}_{\text{females}}=45.7 \pm 4.5\%$), as revealed from an ANOVA analysis ($F_{1,34}=10.686$, $P=0.002$), yet there were no differences among field sites ($F_{4,34}=1.309$, $P=0.286$; Fig. 3A), and the interaction between terms was not significant ($F_{1,34}=1.796$, $P=0.189$).

Mean Hb for all animals was $16.8 \pm 2.7 \text{ g dl}^{-1}$, and it was negatively related to body mass ($R^2=0.196$, $P=0.003$, Fig. 2B).

Table 1. Total body oxygen stores, mass-specific oxygen stores partitioned by compartment (blood, muscle and lungs) and body mass of *Otaria byronia*

| Site | Total oxygen stores (ml O ₂) | Mass-specific oxygen stores (ml O ₂ kg ⁻¹) | | | | Body mass (kg) |
|-----------------------------|--|---|-------------|-----------|-------------|----------------|
| | | Blood | Muscle | Lung | Total | |
| Northern Chile ($n=10$) | 4525.22±285.13 | 24.92±8.24 | 14.78±3.43 | 6.24±0.04 | 46.03±5.56 | 101.7±0.08 |
| Central Chile ($n=4$) | 3672.13±321.22 | 24.78±5.98 | 11.04±2.22 | 6.27±0.06 | 42.68±7.31 | 88.5±0.07 |
| Southern Chile ($n=9$) | 3724.32±593.63 | 26.12±17.46 | 13.16±1.67 | 6.27±0.08 | 46.53±16.67 | 92.7±0.19 |
| Falkland Islands ($n=10$) | 4011.59±638.00 | 19.46±6.47 | 13.21±0.14* | 6.21±0.04 | 38.84±6.48 | 115.1±0.11* |
| Uruguay ($n=10$) | 4302.09±961.58 | 15.48±3.08 | 12.07±4.49 | 6.18±0.04 | 34.00±5.73 | 129.9±0.12 |

*Simulated values.

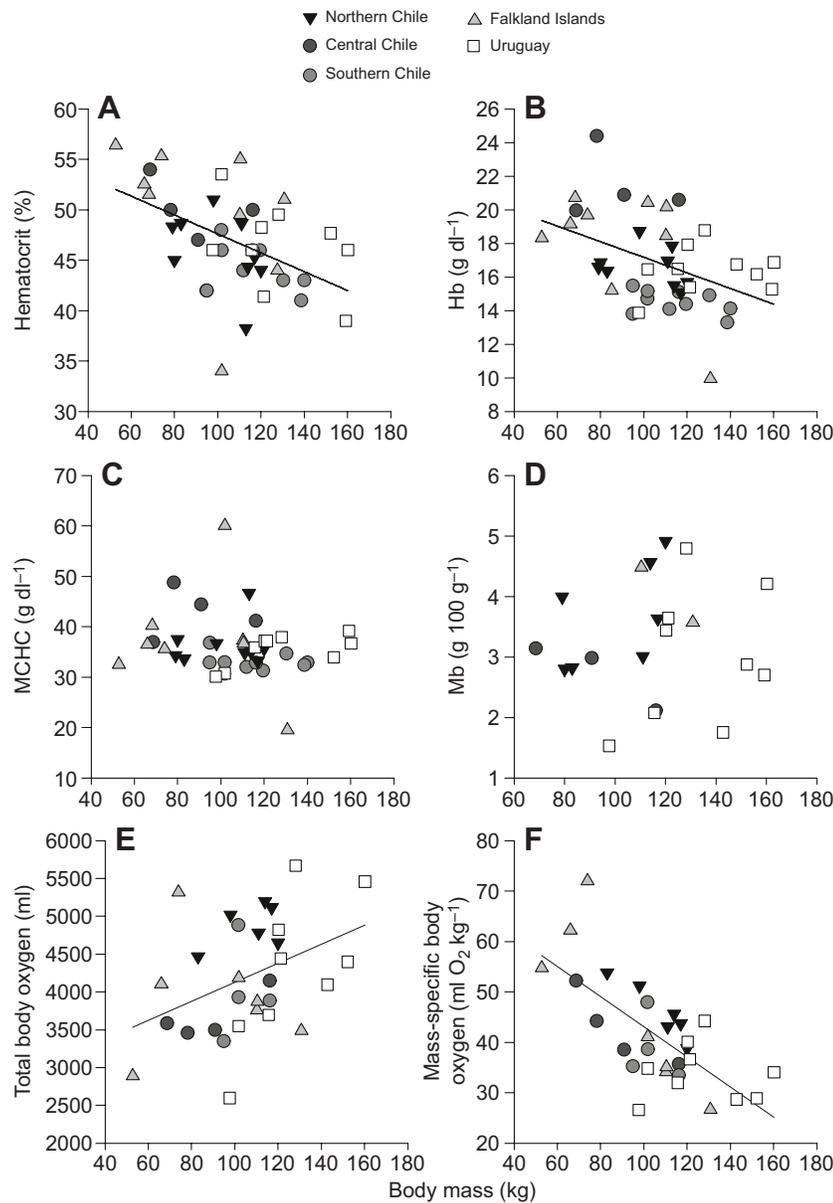


Fig. 2. Relationships between physiological parameters of *Otaria byronia* and individual body mass.

(A) Hematocrit ($n=41$, $R^2=0.235$, $P=0.001$), (B) blood hemoglobin concentration (Hb, $n=42$, $R^2=0.196$, $P=0.003$), (C) mean corpuscular hemoglobin content (MCHC, $n=40$), (D) muscle myoglobin concentration (Mb, $n=21$), (E) total body oxygen stores ($n=30$, $R^2=0.161$, $P=0.028$) and (F) mass-specific body oxygen stores ($n=30$, $R^2=0.486$, $P<0.01$). Missing regression lines indicate no significant relationship with body size.

Females had significantly lower Hb levels (16.1 ± 2.2 g dl⁻¹) than juvenile males (19.6 ± 2.4 g dl⁻¹; ANOVA $F_{1,35}=86.772$, $P<0.001$). The Hb values differed among sites ($F_{4,35}=4.556$, $P<0.005$), with animals from the Falkland Islands having significantly lower Hb values (14.5 g dl⁻¹) than animals from central (21.5 g dl⁻¹) and southern Chile (18.0 g dl⁻¹; Dunn's *post hoc* pairwise test; Fig. 3B), but the interaction between sex and field site was not significant ($F_{1,35}=2.19$, $P=0.45$).

Finally, mean MCHC was 35.38 ± 5.93 g dl⁻¹, which was not related to body mass ($R^2=0.02$, $P=0.348$; Fig. 2C). Similarly, MCHC values did not differ between females (35.43 ± 6.29 g dl⁻¹) and juvenile males (38.5 ± 5.3 g dl⁻¹) ($F_{1,33}=1.933$, $P=0.174$). There were no differences in MCHC among field sites ($F_{4,33}=1.802$, $P=0.152$; Fig. 3C), and the interaction between sex and field site was not significant ($F_{1,33}=0.192$, $P=0.664$).

We found no significant differences in mass-specific blood volumes of sea lions among field sites (ANOVA, $F_{4,28}=2.29$, $P=0.09$; Fig. 4C). Mean mass-specific blood volume did not vary

between females (104.8 ± 28.87 ml kg⁻¹) and juvenile males (121.85 ± 43.54 ml kg⁻¹; *t*-test, $t_{27}=-1.23$, $P=0.23$).

Larger animals had lower mass-specific blood volumes ($R^2=0.1372$, $P=0.023$), which is described as follows for all animals for which we had body mass data: $V_B = -0.779M_b + 195.612$ ($R^2=0.166$, $P=0.023$). The mass-specific blood volume of females is described by: $V_B = -1.318M_b + 261.931$ ($R^2=0.258$, $P=0.013$). And for juvenile males by: $V_B = -0.558M_b + 165.756$ ($R^2=0.059$, $P=0.561$).

Mean blood oxygen content of South American sea lions (all age and sex classes) was estimated to be 22.9 ± 12.5 ml O₂ kg⁻¹. Blood oxygen content varied between adult females (mean: 20.2 ml O₂ kg⁻¹) and juvenile males (mean: 34.1 ml O₂ kg⁻¹; Kruskal–Wallis, $H=9.703$, $P=0.002$), but not across sites (Kruskal–Wallis, $H=8.31$, $P=0.081$; Fig. 3D).

Muscle oxygen stores

Mean muscle Mb concentration of South American sea lions was 3.3 ± 1.0 g Mb 100 g⁻¹. Muscle Mb content was not related to the

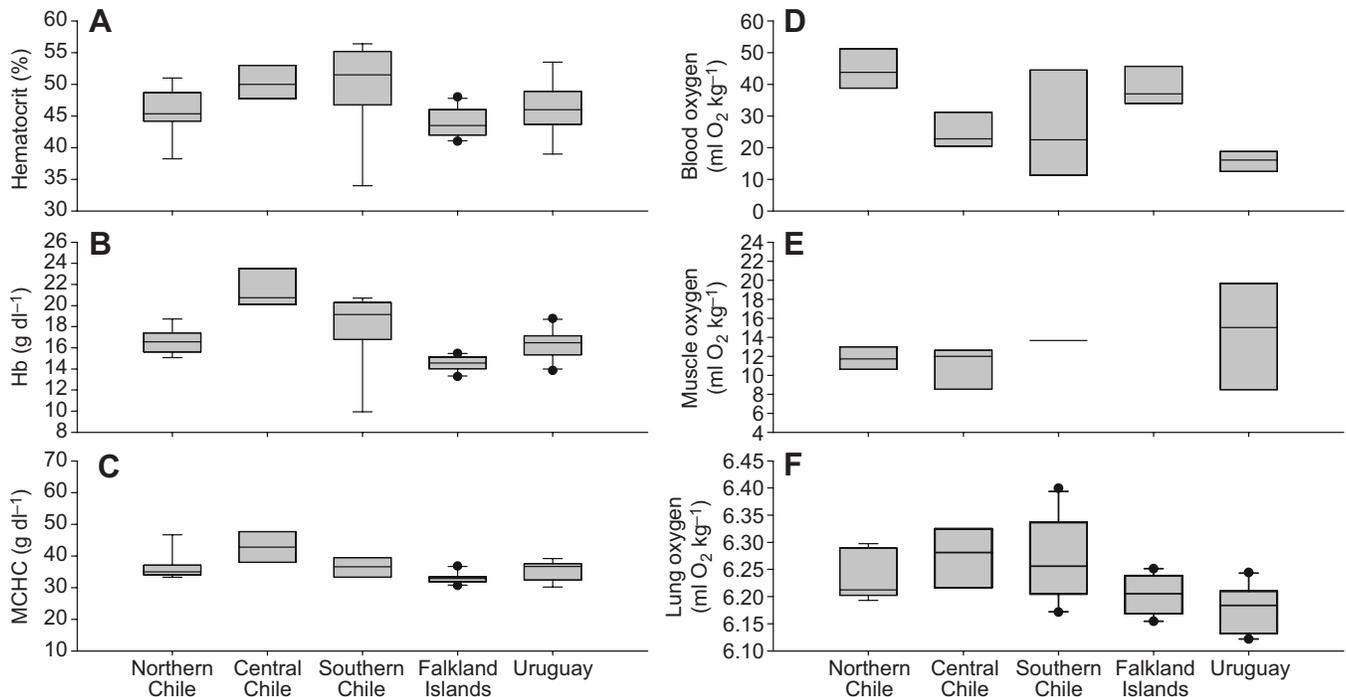


Fig. 3. Regional differences in blood physiological parameters and oxygen storage of *Otaria byronia*. (A) Hematocrit, (B) blood hemoglobin concentration, (C) mean corpuscular hemoglobin content (MCHC), (D) blood oxygen content, (E) muscle oxygen content and (F) lung oxygen content. Data were collected from animals captured in northern Chile ($n=9$), central Chile ($n=4$), southern Chile ($n=10$), the Falkland Islands ($n=10$) and Uruguay ($n=9$).

body mass of sea lions ($R^2=0.005$, $P=0.749$; Fig. 2D). Mb values did not vary between sexes (ANOVA, $F_{1,16}=2.098$, $P=0.167$) or among sites ($F_{3,16}=1.651$, $P=0.217$). Mean mass-specific muscle oxygen content was 12.1 ± 3.8 ml O₂ kg⁻¹ (Fig. 3E).

Total body oxygen stores

Mean total body oxygen stores of South American sea lions was 4209 ± 770 ml O₂, and was positively related to body mass ($R^2=0.161$, $P=0.028$; Fig. 2E), although mass-specific body oxygen (41.1 ± 10.5 ml O₂ kg⁻¹) was negatively related to body mass ($R^2=0.486$, $P<0.001$; Fig. 2F). Total body oxygen stores of South American sea lions were different across the different field sites sampled in this study (Fig. 4B). Our analysis showed significant differences among the different sites sampled in our study (ANOVA, $F_{4,24}=4.934$, $P=0.005$), driven by the low mass-specific body oxygen stores of animals from Uruguay and the Falkland Islands (*post hoc* Holm–Sidak test) (Fig. 4). Differences in mass-specific body oxygen stores were also seen between sexes (ANOVA, $F_{1,24}=27.954$, $P<0.001$), with males in our sample having significantly higher mass-specific total body oxygen stores. The interaction term between sex and site was also significant ($F_{1,23}=5.469$, $P=0.028$), because of the lower total body oxygen stores of males from northern Chile compared with females from the same field site.

We did not find significant differences in total body oxygen stores (ANOVA, $F_{3,19}=1.969$, $P=0.153$), but mass-specific female oxygen stores did vary among sites (ANOVA, $F_{3,18}=4.196$, $P=0.02$; Fig. 4). A *post hoc* Holm–Sidak test revealed that the difference was driven by the higher mean oxygen stores of females from northern Chile compared with females from Uruguay.

Diving behavior

Detailed diving behavior data were not available from animals from central Chile as the instruments deployed there (Wildlife Computer

SPLASH tags) only transmitted summarized data (see Hückstädt et al., 2014), and these data are therefore not comparable with the other sites.

A total of 67,884 dives were identified for animals in this study, which dove to a mean depth of 29.0 ± 34.8 m, reaching a maximum diving depth of 325 m (southern Chile). The mean dive duration for all animals was 109.8 ± 49.8 s, with the longest dive lasting for 724 s (12.07 min). Mean individual maximum diving depth was 134 ± 84 m, while the mean longest dive per individual was 299 ± 101 s.

The diving depth of South American sea lions was significantly different across field sites (Kruskal–Wallis, $H=2544.461$, $P<0.001$), as was dive duration (Kruskal–Wallis, $H=299.363$, $P<0.001$; Fig. 5A). For both variables, all pairwise comparisons among sites showed significant differences (Dunn’s *post hoc* test, all $P<0.05$). Dive duration varied between sexes (Mann–Whitney, $U=31,381,077.5$, $P<0.001$), with adult females diving significantly longer than juvenile males, but there was no difference in dive depth between sexes (Mann–Whitney, $U=35,156,830.0$, $P=0.799$).

The maximum diving depth of each individual differed across sites (Kruskal–Wallis, $H=21.248$, $P<0.001$), mostly because of the shallow dives of animals from Uruguay compared with animals from southern Chile and the Falkland Islands (*post hoc* Dunn’s test; Fig. 5A). Likewise, the diving behavior of the sea lions (pelagic versus benthic), varied across field sites (Kruskal–Wallis, $H=10,397$, $P<0.001$; Fig. 5B). Individuals from northern Chile foraged in the epipelagic zone, as they did not dive deeper than 200 m (boundary between epi- and mesopelagic), using the upper 17% of the water column. Despite diving shallower than 200 m, individuals from Uruguay were predominantly benthic divers, using on average 70% of the water column. Deep-diving (>200 m) sea lions from southern Chile and the Falklands displayed intermediate use of the water column (using 35.5 and 40.3%, respectively), but

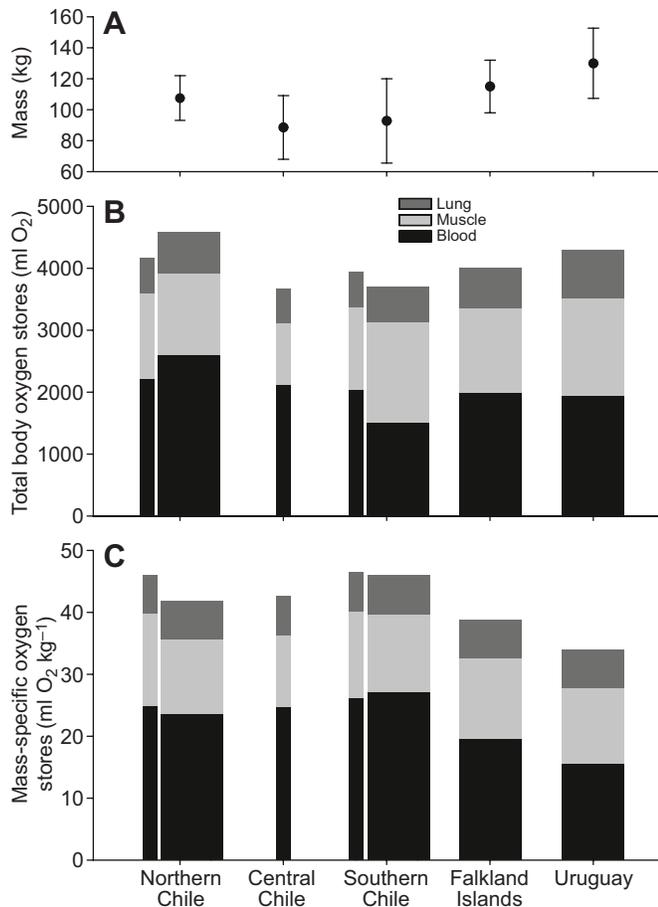


Fig. 4. Regional differences in body oxygen storage of *Otaria byronia*. (A) Body mass, (B) total body oxygen stores by compartment: lungs (dark gray), muscle (light gray) and blood (black) and (C) mass-specific oxygen stores by compartment: lungs (dark gray), muscle (light gray) and blood (black). The narrow bars represent data for juvenile males ($n=9$), and the wide bars correspond to adult female data ($n=21$).

we saw differences in their diving behavior that are more evident when comparing the third quartile (third quartile southern Chile=63%; third quartile Falkland Islands=71%), indicating that animals from the Falklands displayed more benthic behavior than animals from southern Chile (Fig. 5B).

To evaluate the influence of total body oxygen stores on the diving behavior of South American sea lions, we examined the relationship between the third quartile of both diving depth and diving duration of each animal versus their respective mass-specific total body oxygen stores. We chose the third quartile as the distribution of both diving depths and duration as both distributions were greatly skewed toward lower values, as a result of the influence of shallow transit dives. As a result, the mean (or median) does not accurately represent the diving capacity of each animal. When we pooled all individuals together, we did not find any relationship between diving depth or duration and their mass-specific total body oxygen ($\text{ml O}_2 \text{ kg}^{-1}$) (diving depth: $R^2=0.014$, $P=0.625$; diving duration: $R^2 \ll 0.001$, $P=0.929$). Similar negative results were obtained when evaluating the relationships between both variables and total body oxygen stores (ml O_2), as well as when we modeled the relationships with specific blood physiology parameters, such as blood volume, Hct, Hb, Mb and compartment-specific oxygen stores (blood, muscle and lungs; data not shown here).

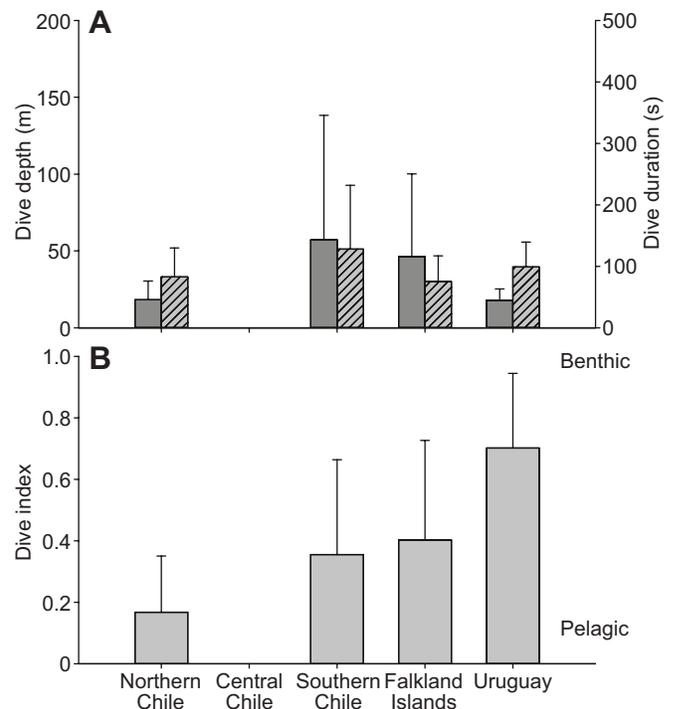


Fig. 5. Regional differences in patterns of diving behavior of *Otaria byronia*. (A) Differences in diving depth (gray bars) and diving duration (striped bars), and (B) dive index, indicating relative use of the water column. The index represents the ratio between diving depth and bathymetric depth. Larger values of this index indicate benthic behavior, whereas lower values indicate pelagic behavior (epi- or mesopelagic).

Calculated aerobic dive limit

Following Weise and Costa (2007), we estimated both minimum and maximum cADL, using swimming and resting metabolic rate, respectively, as reported by Dassis et al. (2012). South American sea lion minimum cADL was 2.22 ± 0.66 min, while maximum cADL was 4.59 ± 1.35 min ($n=21$). Male sea lions had significantly higher cADLs than females (Kruskal–Wallis, $H=9.293$, $P=0.002$) but cADL did not vary between sites (Kruskal–Wallis, $H=8.775$, $P=0.067$) (Fig. 6, Tables 1, 2).

Few individuals had mean dive durations that exceeded their average minimum cADL, while most fell well below the 1:1 line, implying that these animals rarely exceed their cADL (Fig. 6). Interestingly, animals from Uruguay, which were the largest animals in our sample and foraged benthically, fell on or close to the 1:1 line between dive duration and cADL, with one female (UR0905) exceeding her cADL by an average of 0.65 min (147% cADL). All of the animals from southern Chile exceeded their cADL by an average of 0.54 min or 122% cADL (Fig. 6, Tables 1, 2). Animals from the Falklands fell below the 1:1 line by an average of -0.68 min (67% cADL), as did animals from northern Chile

Table 2. Estimated percentage of dives performed by *Otaria byronia* that exceeds the calculated aerobic dive limit (cADL)

| Site | cADL (min) | Dives exceeding cADL (%) |
|------------------|-----------------|--------------------------|
| Northern Chile | 2.41 ± 0.67 | 8.10 ± 5.22 |
| Central Chile | 1.91 ± 0.28 | ND |
| Southern Chile | 1.76 ± 0.60 | 41.15 ± 25.48 |
| Falkland Islands | 1.31 ± 0.48 | 35.74 ± 10.83 |
| Uruguay | 1.89 ± 0.33 | 48.87 ± 23.14 |

ND, not determined.

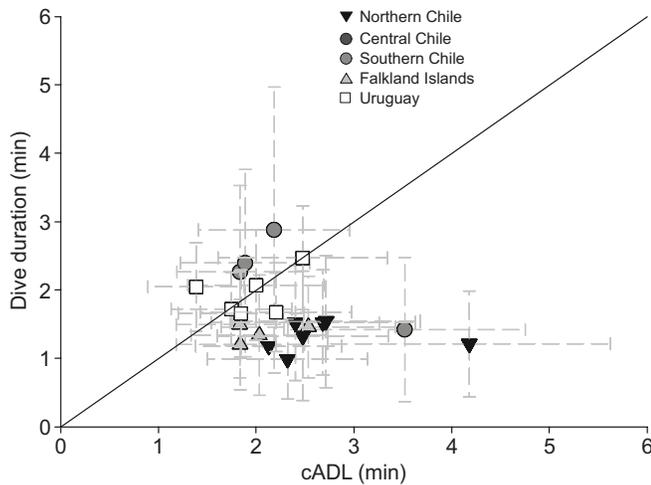


Fig. 6. Relationships between minimum calculated aerobic dive limit (cADL, calculated based on swimming metabolic rate) and dive duration of *Otaria byronia* (n=21). The solid diagonal line represents the 1:1 boundary; data that fall below this line correspond to dives that lasted less than predicted based on estimated individual oxygen storage. Anything above the 1:1 line indicates dives longer than predicted based on estimated individual oxygen storage. Each symbol represents an individual from a different field site, and the dashed lines represent the s.d. in both cADL and dive duration for each individual. Data for animals from central Chile were not available to calculate dive duration.

(average=1.41 min, or 42% cADL). The two individuals with the shortest durations relative to their aerobic dive duration were a juvenile male from southern Chile (SC0904) and a female from northern Chile (NC1107) (Fig. 6).

DISCUSSION

Because of their wide distribution along a diversity of environments on both coasts of South America, sea lions provide a valuable opportunity to study both the behavioral and physiological plasticity of an air-breathing marine predator in different environments. In this study, we measured the oxygen stores and diving behavior of South American sea lions along most of its distribution. Our data showed no clear pattern between total body oxygen stores (a function of body size) and the diving behavior of sea lions across their range. Despite not being able to measure Mb from animals from the Falkland Islands and having to use, instead, simulated values for these individuals, the physiological differences we present in this study are mostly driven by the blood oxygen, and consequently we can include animals from this site in our analyses.

The oxygen storage capacity of an air-breathing marine predator can be closely related to body size, as the larger the individual, the more tissue (blood, muscle and lungs) available to store larger volumes of oxygen (Burns, 1999; Costa et al., 2004; Hassrick et al., 2013; Weise and Costa, 2007). Studies reporting positive relationships of oxygen storage capacity and body mass have typically approached ontogenetic physiological changes, as they have compared smaller, under-developed individuals such as pups and small juveniles with adult individuals (Burns, 1999; Burns et al., 2005; Fowler et al., 2007; Leung et al., 2014; Richmond et al., 2006; Weise and Costa, 2007). As hypothesized, larger South American sea lions had larger total body oxygen stores (Fig. 2E), although this relationship is rather weak ($R^2=0.161$) compared with, for instance, an R^2 of 0.5 (females) and 0.3 (males) in California sea lions *Zalophus californianus* (Weise and Costa, 2007).

Larger animals have a greater physiological capacity for breath-holding diving than smaller animals simply because metabolic rate scales as $mass^{0.75}$ and oxygen stores scale as $mass^{1.0}$ (Costa, 1993). Thus larger sea lions should have greater diving ability than smaller sea lions. It follows that smaller sea lions might exhibit greater mass-specific oxygen carrying capacity to compensate for their smaller size and reduced diving ability. In fact, this pattern has been observed within California sea lions where adult males had higher absolute oxygen stores, while females had higher mass-specific oxygen stores (Weise and Costa, 2007). Similarly, across sea lion species, the Galapagos sea lion, which is the smallest sea lion, has the highest mass-specific oxygen stores of any sea lion, with values comparable to the more proficient diving phocids or true seals (Villegas-Amtmann and Costa, 2010). Finally, the smallest marine mammal, the sea otter *Enhydra lutris*, has mass-specific oxygen stores that are surprisingly high and comparable to those measured for phocid seals and the Galapagos sea lion (Thometz et al., 2015).

Thus, though large body size confers greater diving ability, the increase in oxygen storage may not be the driving or evolutionary constraint in the largest diving mammals. For example, even though male elephant seals have greater diving ability because of their significantly larger mass and oxygen stores than females, they do not dive deeper or longer than females (Hassrick et al., 2010; Le Boeuf et al., 2000). Similarly, the largest mysticete whales do not exhibit particularly long or deep dives as one might predict from their body size alone. Instead, some of the deepest diving air breathers (e.g. beaked whales, elephant seals and emperor penguins) are medium-sized vertebrates (Hindell et al., 1992; Sato et al., 2011; Schorr et al., 2014). Furthermore, it has been proposed that despite its positive effect on body oxygen stores, large body size might actually be disadvantageous for otariids given their high absolute metabolic rate (i.e. elevated oxygen consumption, \dot{V}_{O_2}), which requires higher absolute food requirements (Costa, 1993). Our data in South American sea lions show either negative relationships with body mass (i.e. mass-specific body oxygen content, Hct, Hb), or no significant relationships (MCHC, Mb; Fig. 2).

A possible mechanism to explain the results we observed in the data is that the exposure to diverse habitats and geographical locations could better explain different oxygen storage capacities and diving capability in South American sea lions than body size alone. For instance, the largest animals in our study (individuals from Uruguay) were the shallowest and shortest duration divers, and had the lowest mass-specific total body oxygen stores (mean mass=129.9 kg; dive depth third quartile=26 m; dive duration third quartile=136 s; Figs 2–4), while the deepest and longest duration divers (individuals from southern Chile) had significantly larger mass-specific oxygen volumes, despite being much smaller animals (mean mass=92.7 kg; dive depth third quartile=140 m; dive duration third quartile=232 s). The shallow diving sea lions from Uruguay are limited to waters that do not exceed 50 m in depth (Riet-Sapriza et al., 2013; Rodriguez et al., 2013), while deeper/longer diving animals from southern Chile occupy coastal fjords (which can exceed 300 m in depth) and travel into open waters where they can easily reach and venture beyond the edge of the continental shelf (200 m isobath).

Animals that consistently dive for long durations are known to experience periods of hypoxemia on these dives (McDonald and Ponganis, 2012, 2013; Meir et al., 2009). It has long been known that exposure to hypoxia will increase total body oxygen stores through the production of erythrocytes, Hb and Mb (Knaupp et al., 1992; Maekawa and Kato, 2015; Rodríguez et al., 1999; Zhuang and Zhou, 1998), which could explain the patterns we see in our

data. It follows, then, that these deeper and longer duration dives of animals from southern Chile could be associated with a greater level of hypoxia exposure compared with shallow diving animals from Uruguay, and are therefore a likely contributing factor to hypoxia induced increases in oxygen stores. Similarly, within the same colony of Galapagos sea lions, individuals that made deeper longer dives had greater mass-specific oxygen stores than their shallow diving neighbors (Villegas-Amtmann and Costa, 2010), and California sea lions exhibited increased oxygen stores per kilogram, associated with a seasonal increase in the depth and duration of their dives (Villegas-Amtmann et al., 2012).

As originally hypothesized, the diving behavior of the South American sea lion varied across its distribution. It has been long predicted that the behavior of the species should be different when comparing individuals from the Atlantic coast (relatively shallow waters and wide continental shelf) with individuals from the Pacific coast (deep waters and a narrow continental shelf). Previous studies on the diving behavior along the Atlantic distribution of the species have reported deep and benthic diving for the sea lions (Campagna et al., 2001; Riet-Saprizo et al., 2013; Rodriguez et al., 2013; Thompson et al., 1998; Werner and Campagna, 1995), whereas animals from the Pacific have been proposed as epipelagic foragers (<200 m), although this is mostly based on circumstantial evidence (Hückstädt et al., 2014, 2007; Soto et al., 2004), as diving data had not been collected in the Pacific until the present study. This divergence on the diving behavior matches the results observed in our physiological and diving data (Figs 4–6), and provides further evidence for the hypothesis that the environment could be driving the observed differences in diving performance.

Otariids that display benthic foraging usually exhibit smaller population sizes and lower population growth rates compared with those that feed epi- or mesopelagically (Costa et al., 2004). This dichotomy has been linked to the fact that benthic feeders could be operating at (or near) their physiological limit as estimated based on their ADL (Costa et al., 2004). As shown in our study, South American sea lions can display both benthic and epi/mesopelagic feeding behavior depending on their location (Fig. 5B), and the same general pattern is observed: the population in Uruguay, where animals are almost exclusively benthic, is still decreasing, while the number of sea lions in the Falkland Islands, where we observe a departure from benthic foragers with animals also feeding in the water column, is showing signs of recovery (Baylis et al., 2015b; DINARA, 2012). However, the populations of sea lions along the Chilean coast (predominantly epi- and mesopelagic) are much larger and, when facing catastrophic events such as El Niño, can recover much faster (Crespo et al., 2012).

Although physiological capability is an important factor that determines what an animal can do, differences in environment and behavior also play a role in determining the final performance of an animal. The differences observed between populations of benthic and pelagic feeding sea lions may also be related to the dissimilar efficiency between these two feeding behaviors. In marine ecosystems, benthic prey rarely form aggregations, and predators have to focus on chasing individual prey items that, despite being relatively larger in size and predictable to some extent, might not satisfy their energy requirements. Epi- and mesopelagic prey, in contrast, can form large aggregations (although unpredictable in occurrence), which offer the opportunity for the predator to easily feed to satiation (a behavior observed in several species of sea lions) (Costa, 1991). Furthermore, benthic prey usually have intermediate levels of

energy, whereas pelagic prey can have either high or low energetic content but tend to be much higher in abundance and density (Anthony et al., 2000). Hence, it is possible that feeding pelagically (epi- and mesopelagically) could be a more efficient strategy for the population, as when individuals find prey aggregations these generally offer a large biomass and thus would satisfy the metabolic demands of the sea lions, even in cases of relatively low-energy prey. Indeed, South American sea lion pups from females that feed pelagically have been found to exhibit higher growth rates than pups from animals that exhibit a benthic diet (Drago et al., 2010). These differences in energy content and availability between different foraging patterns could become even more problematic for species and populations that need to make deep dives to forage on benthic or demersal prey, such as New Zealand sea lions that do push their cADL (Costa and Gales, 2000), and future studies should address whether these ecological differences could be driving the different population trends.

This difference in population characteristics between benthic and pelagic feeders is not exclusive to otariids, as we can see commonalities when comparing with other species of pinnipeds and seabirds. For instance, crabeater seals, *Lobodon carcinophaga*, the most numerous species of pinniped in the world, feed almost exclusively on Antarctic krill, *Euphausia superba* (Hückstädt et al., 2012), despite the relatively low energetic content of this prey item compared with fish. Likewise, the Antarctic fur seal, *Arctocephalus gazella*, and Cape fur seal, *A. pusillus pusillus*, both of which also feed on pelagic prey (Antarctic krill and myctophids, and small pelagic fish, respectively), have both recovered after cessation of their exploitation, reaching population levels near or at carrying capacity (Forcada et al., 2005; Huccke-Gaete et al., 2004; Magera et al., 2013; Wickens and York, 1997). In contrast, the Critically Endangered Hawaiian monk seal, *Neomonachus schauinslandi*, and the Australian sea lion, *Neophoca cinerea*, are among the best examples of benthic foragers with low to very low population numbers that continue to experience reduction in their populations (Antonelis et al., 2006; Costa and Gales, 2003; Parrish et al., 2002; Shaughnessy et al., 2011).

Another argument that supports foraging efficiency (as opposed to solely diving physiology) as an additional mechanism to explain contrasting population trends comes from one of the best studied deep diving marine mammals: the northern elephant seal, *Mirounga angustirostris*. Adult female elephant seals are mesopelagic foragers (200–1000 m depth) of myctophid fish and squid and their population has shown a remarkable recovery, with growth rates that have approximated 20% per year after having experienced a large decrease in their population (which reached <20 individuals) as a result of human exploitation. The mean dive duration of elephant seals is well within the reported cADL values for the species, despite being capable of routinely performing dives up to three and four times their reported cADL without any effects (Hassrick et al., 2010).

Our study highlights the importance of ADL in determining how and when animals push their physiological capabilities and thus limit the ability of their populations to grow. Our work indicates that the physiology of air-breathing diving predators is not fixed, but that it can be adjusted, to a certain extent, depending on the ecological setting and or habitat. These adjustments can be thought of as a ‘training effect’: as the animal continues to push its physiological capacity through greater hypoxic exposure, its breath-holding capacity increases. Further, some authors mistakenly use ADL as a fixed threshold beyond which animals enter a negative energy

balance, when in reality it should not be considered as a set value, particularly considering the uncertainty in its estimation. For instance, new evidence suggests that cADL is often overestimated as up to 10% of the blood Hb can be bound to endogenous carbon monoxide instead of oxygen (Tift et al., 2014). Hence, it is not surprising that we see that individuals from different species can routinely perform dives that exceed this likely overestimated physiological limit, such as South American sea lions in southern Chile (Fig. 6). We propose that the linkage between foraging behavior and demographics is a complex interaction between physiology, diet, prey distribution and habitat. Future studies should investigate the role that the distribution, abundance and energetic content of their prey play in limiting the population growth of South American sea lions, which could explain the opposing trends we observe for the species across its range.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceived and design the study: L.A.H. and D.P.C. Performed the experiments: L.A.H., D.P.C., F.R.-S., V.F.-T., A.M.M.B., R.A.O., J.P.Y.A., M.S., M.S.-C. and D.P.C. Analyzed the data: L.A.H. and M.S.T. Contributed reagents/materials/analysis tools: D.P.C., A.M.M.B., J.P.Y.A., M.S. and J.M.B. Wrote the paper: L.A.H., M.S.T. and D.P.C.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.138677.supplemental>

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