



Seasonal variation in blood and muscle oxygen stores attributed to diving behavior, environmental temperature and pregnancy in a marine predator, the California sea lion

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ABSTRACT

Survival depends on an animal's ability to find and acquire prey. In diving vertebrates, this ability is directly related to their physiological capability (e.g. oxygen stores). We studied the seasonal variation in oxygen stores, body temperature and body condition in California sea lions (*Zalophus californianus*) (CSL) as a function of seasonal variation in temperature, primary productivity, diving behavior and reproductive stage. During summer, blood oxygen stores were significantly greater and muscle oxygen stores were significantly lower than in winter. Total oxygen stores, body condition and body temperature did not change between seasons but variations in body temperature were greater during summer. Changes in oxygen stores are partly attributed to diving behavior, temperature and pregnancy that could increase oxygen consumption. Blood and muscle oxygen stores appear to be influenced by reproductive state. Blood oxygen stores are more likely influenced by diving behavior and temperature than muscle oxygen stores.

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1. Introduction

The physical environment changes at different temporal scales; over centuries, decades, years and seasonally within a year. Two of the most important features of seasonal change are temperature and light, which directly affect abundance, distribution, physiology and behavior of prey and their predators. Many taxa deal with an unsuitable seasonal change by migrating to a more suitable environment, such as butterflies, whales and birds (Urquhart and Urquhart, 1978; Mate et al., 1999; Tremblay et al., 2006) or by means of hibernation and aestivation as seen in bears, badgers and frogs (Schooley et al., 1994; Tanaka, 2006; Tracy et al., 2007). Colony breeding species are central place foragers and for some species like the California sea lion, *Zalophus californianus* (CSL) (Lesson, 1828) females are constrained by the need to remain with their pup throughout the year and thus are not able to migrate to a more suitable habitat.

Survival of a population is influenced by its foraging success. In diving vertebrates such as sea lions, foraging success is directly related to the distribution, quality and availability of prey coupled with their

physiological capability to dive and acquire that prey. Important determinants of the physiological capacity to dive and thus the time an animal can forage are its oxygen stores, its diving metabolic rate and the extent to which the oxygen stores can be depleted (Costa et al., 2001; Meir et al., 2009). In sea lions and fur seals oxygen to support aerobic metabolism during diving is stored in the lungs (10–21%), blood (41–70%) and muscle (20–43%) (Kooyman, 1989; Ponganis, 2011).

Oxygen stores have been measured in a variety of diving vertebrates, including penguins, seals, dolphins, moles, shags, turtles (Kooyman and Ponganis, 1990; Luttcavage et al., 1992; Ponganis et al., 1993; McIntyre et al., 2002; Noren et al., 2002; Burns et al., 2005; Cook et al., 2008; Hassrick et al., 2010), sea lion and fur seals (Lenfant et al., 1970; Horning and Trillmich, 1997; Costa et al., 1998, 2001; Richmond et al., 2006; Fowler et al., 2007; Weise and Costa, 2007; Villegas-Amtmann and Costa, 2010). However, only one study has examined the seasonal changes in oxygen stores concurrently with changes in diving behavior (Villegas-Amtmann and Costa, 2010). In this study individual Galapagos sea lions (GSL) (*Zalophus wollebaeki*) exhibited three different diving patterns and each was associated with a different amount of stored oxygen. The longest diving individuals had the highest oxygen stores, while the shortest duration divers had the lowest oxygen stores. Further, while the diving behavior didn't change between seasons, the blood and muscle oxygen stores did (Villegas-Amtmann and Costa, 2010).

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California sea lions are widely distributed from the western coast of North America, from the Gulf of Alaska to the south of Mexico, including the Gulf of California (Maniscalco et al., 2004; Aurioles and Trillmich, 2008). The population in the Gulf of California has been estimated at around 30,000 individuals (Szteren et al., 2006) of the 355,000 global population (Aurioles and Trillmich, 2008). In the Gulf of California, sea lions encounter a wide range of environmental temperatures within a year that influence primary productivity and prey availability. Sea surface temperature (SST) in the Gulf of California ranges annually from 15 to 31 °C and chlorophyll-*a* ranges from 0.3 to 6.0 mg/m³ (<http://coastwatch.pfeg.noaa.gov/>) between the coldest (winter) and warmest (summer) seasons of the year.

We previously found that CSLs change their diving behavior between these contrasting seasons, exhibiting greater variability during the warm and less productive season (Villegas-Amtmann et al., 2011). In addition to these seasonal changes in diving behavior, SST and primary productivity, the reproductive pattern of CSLs varies with females giving birth followed by copulation and delayed implantation during the warm season (summer) with the active growth of the fetus occurring during the cold season (winter) (Odell, 1975).

Here we examine the seasonal variation of oxygen stores, core body temperature and body condition in California sea lions from Granito Island, Gulf of California, Mexico during the two most contrasting seasons of the year, summer and winter. We hypothesized that CSLs would exhibit higher oxygen stores, core body temperature and lower body condition during the warm season and that these differences would be associated with the changes in diving behavior (deeper and longer dives), warmer ambient and SST, lower primary productivity and reproductive stage (~1 month after giving birth).

2. Materials and methods

2.1. Field site and capture procedures

Measurements were carried out during a warm season, the end of July 2005, and a cold one, the end of February 2007 at Granito Island (29.55° N, 113.54° W) in the Gulf of California, Mexico. During the warm season we captured 10 lactating female CSLs, which were suckling small pups, 1 to 2 months after the peak pupping season. During the cold season we captured 11 lactating female CSLs, with older pups (8 to 9 months after peak pupping season). Due to the timing of the field effort the lactating females in each season were not the same individuals.

Sea lions were captured with hoop nets and anesthetized with isoflurane administered using a portable anesthesia machine (10 L/min with a 3 L rebreathing bag). Anesthesia was induced while the animal was inside the net by placing a plastic mask (modified highway road cone) on the sea lions' snout with isoflurane delivered at 4 to 5% in 100% oxygen. Induction time varied from 5 to 25 min depending on the animals' excitement and amount of apneas. Intravenous propofol at a dose of 1 mg/kg was used as an induction agent for some animals 5 min after the start of the isoflurane induction. Animals were intubated using a large laryngoscope blade (35 cm) and endotracheal tubes with an internal diameter of 14–18 mm. The anesthesia was maintained with 1.5 to 2% of isoflurane (Gales and Mattlin, 1997; Parás, 2008).

Samples were taken while the animal was anesthetized and the instruments were introduced and attached to measure core body temperature and locate the animals. The recovery of the instruments was facilitated by locating the animals using small VHF radio transmitters (Sirtrack; Havelock North, New Zealand) or ARGOS linked PTTs (Wildlife Computers, Redmond WA, USA).

Core body temperature was obtained by equipping CSLs with heart rate-stomach temperature recorders (HTR) (set to sample only the stomach temperature) and stomach temperature transmitters (STT) (Wildlife Computers). We deployed 10 HTRs and STTs in 2005 and 8 in 2007, which were attached to the animal's back and placed in the

animal's stomach respectively. In the STT, 4 thermistors monitor the temperature, and the coldest measured temperature is relayed to the HTR. The STT "pings" a radio-pulse of 5 kHz. The ping-rate is temperature-dependent; the warmer the temperature, the faster the ping-rate. The STT is calibrated to measure 0 to 50 °C, with a resolution of 0.1 °C, and an accuracy of approximately ±1 °C (Wildlife Computers). The STT is eventually defecated or regurgitated (lost to the environment) and HTRs stop recording data at that time.

We mounted the instruments on mesh netting and glued them to the dorsal pelage of the lower back (HTR) and between the shoulders (PTT and VHF) of the animals using a 5 minute quick set Locktite epoxy (Locktite Quickset™, Henkel Corp., Dusseldorf, Germany).

The total weight of the instruments attached was approximately 230 g (~0.23% of the animal's mass). We weighed animals in a sling using a tripod and a 250 kg (±0.1 kg precision) capacity digital scale and took standard length measurements using a measuring tape.

The equipment was either removed by recapturing and physically restraining the animals without anesthesia or was recovered on the rookery around the rocks in the inter-tidal zone after falling off the animal during the molt (possibly at the beginning of August). Epoxy mounts fall off during the animals' annual molt.

This research was approved by the CARC (Chancellor's Animal Research Committee) at University of California, Santa Cruz, CA, USA (COST 00.05).

2.2. Physiological sample collection and analyses

We collected blood samples from CSLs from the caudal-gluteal vein for hematocrit (Hct) and hemoglobin concentration ([Hb]) determination in 2005 (n = 10) and 2007 (n = 11). Blood samples used to determine Hct were taken prior to induction of anesthesia because Hct values decline with time under anesthesia (Ponganis et al., 1992; Costa et al., 1998). Hct, [Hb], plasma volume (Pv) and blood volume (Bv) were determined following previously described methods (Swan and Nelson, 1971; Foldager and Blomqvist, 1991; El-Sayed et al., 1995; Costa et al., 1998; Villegas-Amtmann and Costa, 2010).

Muscle myoglobin concentration ([Mb]) was determined following the method of Reynafarje (1963) in muscle samples obtained using a 6 mm dermal biopsy punch (MilteX, Inc.) from the primary locomotion muscle, *supraspinatus* muscle, above the pectoral flipper. Muscle samples were stored at –80 °C until analysis.

We calculated the total body oxygen stores by adding blood, muscle and lung oxygen stores (Lenfant et al., 1970; Kooyman et al., 1983; Ponganis et al., 1997; Costa et al., 2001) following the methods of Fowler et al. (2007). Blood and muscle oxygen stores were obtained in this study and lung oxygen stores were derived by allometric estimates of lung volume for otariids (Kooyman, 1973; Kooyman and Sinnett, 1982; Costa et al., 2001). The body condition of CSL was calculated by dividing mass/standard length (Arnould, 1995; Kotiaho, 1999).

Core body temperatures (°C) were analyzed using a software provided by the manufacturer (HexDecode and 3M programs, Wildlife Computers) and seasonal means were compared using a two-sample Student's *t*-test.

The females sampled during the cold season were examined by a wildlife veterinarian (Paras-Garcia) in the field to determine if they were pregnant. Females were diagnosed as pregnant when they exhibited a considerably visible and palpable distended uterus and by palpation of the fetus, which can only be achieved at an advanced pregnancy state. Pregnancy was later confirmed by performing hormone analysis. We assayed serum samples in duplicate for progesterone using a commercial radioimmunoassay kit (Coat-A-Count Progesterone; Siemens, Los Angeles, CA, USA) and a double-antibody radioimmunoassay (MP Biomedicals, LLC, Orangeburg, NY, USA) for total estrogens. Radioactivity of the bound portion was determined using a gamma counter (Gamma C12; Diagnostic Products Corporation, Los Angeles, CA, USA). The radioimmunoassay was performed as per manufacturer

instructions and following the methods in Villegas-Amtmann et al. (2009) and Greig et al. (2007).

We tested data for normality using a Kolmogorov–Smirnov one sample test and for homogeneity of variance. Comparisons between seasons were performed using *t*-tests or when data were not normally distributed using Kruskal–Wallis tests (K–W).

3. Results

California sea lions exhibited changes in their oxygen stores between seasons. Blood oxygen store components measured: [Hb] (*t*-test, *t* = −2.4, *df* = 19, *P* = 0.03), Bv (*t*-test, *t* = −2.49, *df* = 18, *P* = 0.02) and Pv (*t*-test, *t* = 5.07, *df* = 18, *P* < 0.00) were significantly greater during the warm season compared to the cold season. In contrast, muscle oxygen stores measured as [Mb] (*t*-test, *t* = 2.43, *df* = 18, *P* = 0.02) were significantly lower during the warm season compared to the cold season. Hct values, mass and body condition index were not significantly different between seasons (Table 1, Figs. 1 and 2).

Mass specific total oxygen stores were not significantly different between seasons, but the relative contribution of the different oxygen stores was significantly different. During the warm season 12.5%, 55.2% and 32.3% of the total oxygen was stored in the lung, blood and muscle, while during the cold season it was 13.7%, 44.3% and 42% respectively. Mean mass specific total blood oxygen stores were significantly greater during the warm season (26.5 ± 8.7 mL/kg) compared to the cold season (19.4 ± 4.2 mL/kg, *t*-test, *t* = 2.29, *df* = 17, *P* = 0.03). Mean mass specific total muscle oxygen stores were significantly greater during the cold season (18.4 ± 2.8 mL/kg) than during the warm season (15.5 ± 2.3 mL/kg, *t*-test, *t* = −2.46, *df* = 17, *P* = 0.02). Mean mass specific lung oxygen stores were not significantly different between seasons (6.0 ± 0.05 and 6.0 ± 0.03 mL/kg for the warm and cold seasons respectively) (Fig. 2).

We recovered data from 4 HTRs in 2005 that sampled body temperature from 4 h to 2 days and 4 HTRs in 2007 that sampled temperature from 4 h to 1 day. STTs were expelled by one sea lion in 2005 and all of them in 2007 before the initiation of diving and thus data are mostly from when animals were on land (less than 13% of temperature data are from when animals were at sea). In order to remove the effect of prey ingestion or diving activity on core body temperature, records were filtered to eliminate sudden changes in temperature that occurred over a period of no longer than 2 min. Mean core body temperature was determined for each individual and was not significantly different between seasons. However, the variance among individuals (calculated

from the body temperature means of each individual) and the mean of the individual variances (±SD) (calculated from all body temperature data of each individual) was greater during the warm season (0.30 and 1.4 ± 2.2 respectively) compared to the cold one (0.05 and 1.03 ± 1.4 respectively) (Fig. 3).

Mean progesterone concentration (±SD) was higher during the cold season (February) (7.6 ± 5.2 ng/mL, *n* = 10) than during the warm season (July) (4.8 ± 2.9 ng/mL, *n* = 11). Seven out of 11 females were diagnosed as pregnant by veterinary physical examination and 6 females presented progesterone concentrations ≥ 7.44 ng/mL. Greig et al. (2007) found that progesterone concentrations of 7.44 ng/mL were indicative of California sea lions during mid-pregnancy. The mean progesterone concentration of our females was 11.8 ± 2.8 ng/mL (*n* = 6) (Fig. 4). Only 2 out of 10 females presented progesterone concentrations ≥ 7.44 ng/mL during the warm season and none were diagnosed as pregnant by veterinary examination (Fig. 4).

The mean estrogen concentration was significantly greater during the cold season (1.1 ± 0.7 ng/mL, *n* = 11) than during the warm one (0.3 ± 0.2 ng/mL, *n* = 10, *t*-test, *t* = 2.09, *df* = 19, *P* < 0.00). The mean estrogen concentration of females that exhibited progesterone concentrations ≥ 7.44 ng/mL during the cold season was 1.3 ± 0.8 ng/mL (*n* = 6) and the mean estrogen concentration of females that exhibited progesterone concentrations < 7.44 ng/mL during the cold season was 0.7 ± 0.3 ng/mL (*n* = 5) (Fig. 4).

4. Discussion

CSL blood and muscle oxygen stores measured in this study are comparable to that of previous studies on the same species (Hedrick and Duffield, 1991; Ponganis et al., 1997; Weise and Costa, 2007). However, blood volumes for the warm season in this study are somewhat larger than those in previous studies possibly due to sampling periods and sex/mass differences. Hedrick and Duffield (1991) only sampled 2 sea lions and sex or mass was not specified. Sea lions in the Ponganis et al. (1997) study were immature animals and were smaller (34–42 kg) than the females in this study (65–109 kg). Smaller individuals have proportionally lower oxygen storage capacity (Costa et al., 2004; Weise and Costa, 2007). The Weise and Costa (2007) sampling period included 2 seasons (Sept–Oct. and March) and their reported blood volumes are comparable to our values from the cold season (late February). The relative distribution of oxygen in the tissues of CSL is also consistent with that reported for other California sea lions (Kooyman and P.J., 1998; Weise and Costa, 2007).

Table 1
Oxygen stores of female California sea lions (*Zalophus californianus*) from Granito Island, Mexico.

ID	Hct %	[Hb] g/dL	[Mb] g/100 g	Bv mL/kg	Pv mL/kg	Total O ₂ stores mL/kg	Mass (kg)	BCI	ID	Hct %	[Hb] g/dL	[Mb] g/100 g	Bv mL/kg	Pv mL/kg	Total O ₂ stores mL/kg	Mass (kg)	BCI
Warm season									Cold season								
GF01 ^a	49	16.8	4.0	152.1	77.6	51.1	92.2	0.55	GFC01	50	15.0	5.4	86.8	43.4	42.0	81	0.49
GF02	50	19.6	x	162.8	81.4	x	100.6	0.53	GFC02 ^a	49	15.8	4.7	125.1	63.8	47.1	78.2	0.49
GF03	48	17.7	3.2	128.9	67.0	44.8	96.6	0.54	GFC03	49	15.7	5.4	144.5	73.7	53.3	68.8	0.43
GF04	57	20.6	4.1	198.3	85.3	70.2	78.6	0.46	GFC04 ^a	51	16.1	4.2	x	x	x	85.6	0.52
GF05 ^a	41	14.4	4.5	127.3	75.1	44.4	64.8	0.43	GFC05 ^a	49	15.8	4.2	112.0	57.1	42.6	80.4	0.48
GF06	47	17.0	3.7	91.0	48.2	38.2	108.8	0.62	GFC06 ^a	53	17.1	5.3	107.4	50.5	48.0	86.8	0.50
GF07	43	15.0	3.5	125.4	71.5	40.8	93.2	0.56	GFC07 ^a	48	14.5	3.5	108.8	56.6	37.5	87	0.51
GF08	51	17.6	4.9	117.0	57.3	49.0	105.4	0.63	GFC08	54	16.6	4.7	123.5	56.8	48.2	89.8	0.53
GF09	50	17.9	3.7	128.4	64.2	47.0	93	0.56	GFC09 ^a	47	17.3	4.6	116.0	61.5	47.3	78.2	0.49
GF10	53	17.8	3.2	134.7	63.3	46.4	97.2	0.57	GFC10 ^a	49	14.4	3.5	83.3	42.5	33.2	107	0.61
									GFC11 ^a	51	17.0	4.5	78.3	38.3	39.0	84.8	0.49
Mean	48.9	17.5	3.9	136.6	69.1	48.0	93.0	0.5	Mean	50.0	15.9	4.5	108.6	54.4	43.8	84.3	0.5
± s.d.	4.6	1.9	0.6	28.9	11.4	9.2	12.9	0.1	± s.d.	2.1	1.0	0.7	20.8	10.9	6.1	9.4	0.04

Note. Hct: hematocrit, [Hb]: hemoglobin concentration, [Mb]: myoglobin concentration, Pv: plasma volume, Bv: blood volume and BCI: body condition index. Warm season: Jul–Aug 2005 and cold season: Feb–Mar 2007.

^a Pregnant females based on [progesterone] > 7 ng/mL (Greig et al., 2007) and/or field examination.

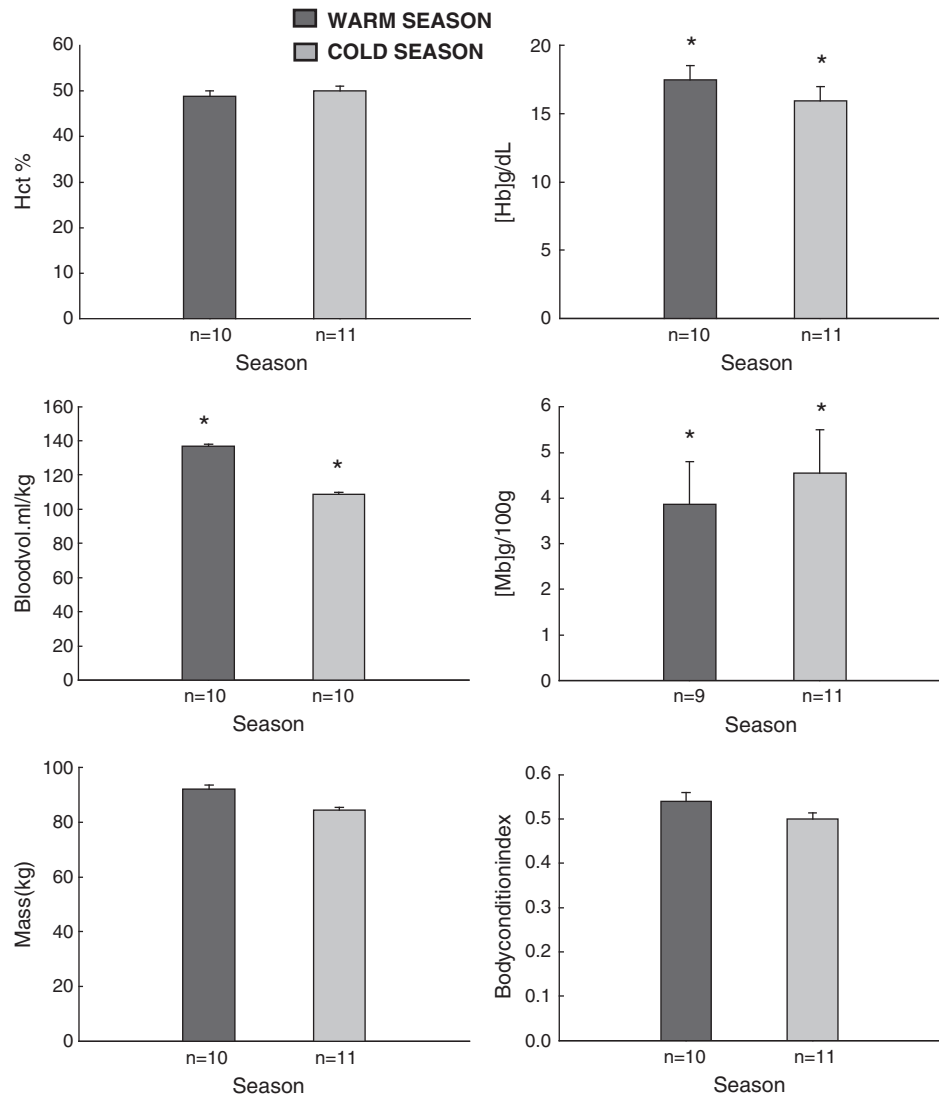


Fig. 1. Mean oxygen stores of California sea lion females (*Zalophus californianus*) from Granito Island, Mexico: hematocrit (Hct), hemoglobin concentration ([Hb]), blood volume (Bv), myoglobin concentration ([Mb]), mass and body condition index (BCI – mass/std. length) \pm SE during a warm (Jul–Aug 2005) and a cold (Feb–Mar 2007) season. * = statistical significance (two-sample Student's *t*-test, $P < 0.05$).

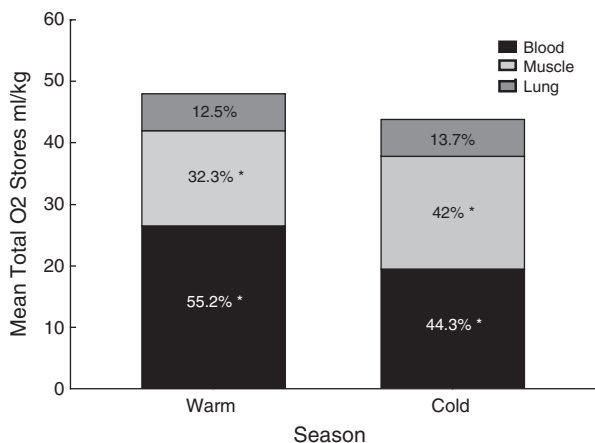


Fig. 2. Total oxygen stores showing their relative proportion in blood, muscle and lung for adult female California sea lions (*Zalophus californianus*) from Granito Island, Mexico during a warm (Jul–Aug. 2005, $n = 9$) and a cold (Feb–Mar. 2007, $n = 10$) season. * = statistical significance (two-sample Student's *t*-test, $P < 0.05$).

4.1. Diving physiology and behavior

CSL blood and muscle oxygen stores changed between seasons; showing contrasting patterns. During the warm season blood oxygen stores were significantly greater and muscle oxygen stores significantly lower (assuming a constant muscle mass supported by morphometric measurements) than during the cold season when this pattern was reversed (Fig. 1). The diving behavior of these sea lions changed between seasons, with females diving deeper and longer during the warm season (Villegas-Amtmann et al., 2011). Such a change in diving behavior may be expected if there was a concurrent change in total oxygen stores, but total oxygen stores were not significantly different between seasons (Fig. 2). However, a change in diving behavior does not necessarily dictate a change in diving physiology as the animal may not have been operating at or near its aerobic dive limit. Thus, a change in diving behavior to longer/deeper dives does not necessarily require an increase in total oxygen stores. Nevertheless, the distribution of oxygen stores did vary significantly between seasons and blood oxygen stores were greater during the warm and less productive season concurring with sea lions diving deeper and longer (Villegas-Amtmann et al., 2011).

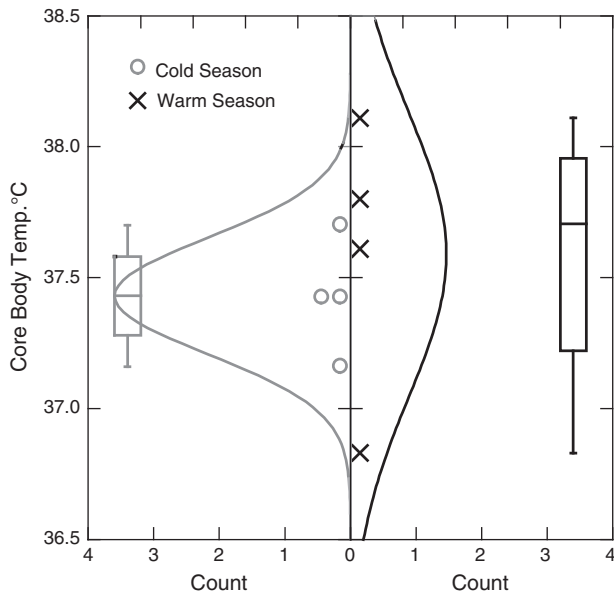


Fig. 3. Frequency distribution and box plots of the mean core body temperature for adult female California sea lions (*Zalophus californianus*) from Granito Island, Mexico showing the difference in variance between a warm (Jul–Aug. 2005) and a cold (Feb–Mar. 2007) season.

During the warm season, warmer water temperatures and lower productivity might shift the distribution of sea lion prey deeper into the water column. The diet of CSLs at Granito Island varies seasonally. During the warm season they feed primarily on Pacific cutlassfish (*Trichiurus lepturus*), a benthopelagic fish (Riede, 2004), while during the cold season they feed mainly on sardine (*Sardinops caeruleus*) and sanddab (*Citharichthys* sp.), pelagic and demersal fish respectively (Lamb and Edgell, 1986; Garcia-Rodriguez and Auriol-Gamboa, 2004; Riede, 2004). The seasonal variation in diet is consistent with the observed changes in diving behavior and the enhanced diving capability during the warm season. During the summer when the animals are feeding on deeper benthic prey they require greater blood oxygen stores to enable longer dives. Whereas during the winter they are feeding on pelagic and demersal fish that require shorter dives (Costa et al., 2004)

Enhanced blood oxygen stores by dive-conditioning have been observed in muskrats (*Ondatra zibethicus*), harbor seals (*Phoca vitulina*) and tufted ducks (*Aythya fuligula*) (Kodama et al., 1977; Stephenson

et al., 1989; MacArthur et al., 2003). Therefore, changes in the distribution of blood oxygen stores observed in this study can be partly attributed to the diving behavior changes driven by prey distribution shifts due to seasonal change (water temperature).

4.2. Physiology and environmental temperature

CSLs encountered a broad range of sea surface temperatures (SST) (14–34.5 °C) (means of minimum and maximum SST) as measured by time–depth recorders deployed on these animals for a different study and air temperature (17–39 °C) between seasons (IBAJANOR1 weather station, wunderground.com). As expected, the mean SST was significantly greater during the warm season (28.8 ± 1.1 °C) than during the cold one (17.5 ± 0.9 °C) (Villegas-Amtmann et al., 2011).

Stomach temperature data was obtained almost exclusively on land due to the STTs being expelled before the diving activity began. CSL body temperature on land as approximated by stomach temperature measurements was not significantly different between seasons, but its variance was greater during the warm season (0.30) compared to the cold one (0.05) (Fig. 3). On land, the upper critical temperature in CSL beyond which its metabolic rate increases in order to thermoregulate has been defined as 21–22 °C (Matsuura and Whittow, 1973). In water, the upper critical temperature has not been defined yet; but in water temperatures of up to 30–32 °C, sea lions maintained a stable metabolic rate (Matsuura and Whittow, 1973; Liao, 1990). CSLs in this study encountered ambient and SST as high as 41 °C during the warm season (Villegas-Amtmann et al., 2011) that greatly exceeded their defined upper critical temperature on land. Matsuura and Whittow (1973) found that the CSL's core body temperature increased when exposed to ambient temperatures of 32 °C. It may be more challenging for sea lions to thermoregulate under the temperature conditions encountered during the warm season, hence the greater fluctuation in their core body temperature (Fig. 3).

Environmental temperatures that exceed the upper critical temperature also result in an increase in metabolic rate as a consequence of the work required to dump excess heat (Matsuura and Whittow, 1973). An increase in total oxygen stores during the warm season could be partly attributable to an increase in metabolic rate and therefore oxygen consumption to thermoregulate. Although we did not find changes in total oxygen stores, blood oxygen stores ([Hb], Bv and Pv) were significantly higher during the warm season. Significantly elevated hemoglobin levels at warmer temperature regimes have been shown in fish (Houston and Gingrasbedard, 1994). Natural heat acclimatization often results in increases in the circulating blood volume in humans (Bass et al., 1955; Wyndham et al., 1968; Senay and Kok, 1976;

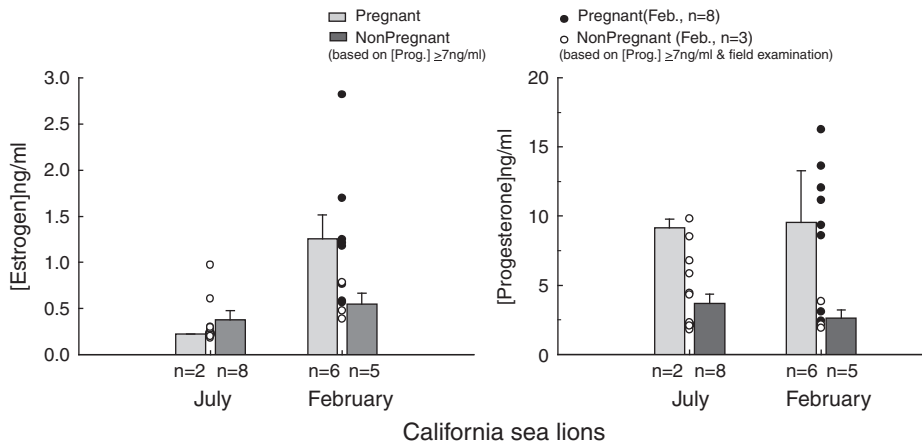


Fig. 4. Mean and individual progesterone and estrogen concentrations (ng/mL ± SE) from adult female California sea lions (*Zalophus californianus*) from Granito Island, Mexico detected as being pregnant (P) and non-pregnant (NP) (based on progesterone concentrations: ≥ 7 ng/mL (Greig et al., 2007)) during July 2005 (P: n = 2, NP: n = 8) and February 2007 (P: n = 6, NP: n = 5). Solid circles indicate pregnant females based on progesterone concentrations ≥ 7 ng/mL and/or diagnosed as being pregnant during field examinations (P: n = 8, NP: n = 3, Feb.2007). Open circles indicate non-pregnant females.

Shapiro et al., 1981). Therefore we can partly attribute the observed changes in blood oxygen stores to environmental conditions (ambient and SST).

4.3. Hormone analyses

Taking into account progesterone concentration values reported in pregnant California sea lions (Greig et al., 2007) and physical examination of the sea lions performed in the field, we can confirm that at least 8 out of 11 females were pregnant during the cold season in February (Table 1, Fig. 4). High progesterone concentrations in 2 females during the warm season in July could have been caused by the increase in circulating progesterone concentration that follows a peak in estrogen concentration (estrus), as seen in Antarctic fur seals (Boyd, 1991a) and several species of seals (Atkinson, 1997). The California sea lion estrus and fertilization period occurs in July (Odell, 1975), thus the high progesterone concentrations were likely due to the normal luteal phase that follows ovulation (Atkinson, 1997).

We found significantly higher concentrations of estrogen during the cold season in February, when sea lions are in a mid to late gestational phase than during the warm season in July when females had given birth recently, ovulated, and entered the normal embryonic diapause (Fig. 4). Higher estrogen concentrations are expected in pregnant females as estrogen levels rise steadily during pregnancy (Reijnders, 1990). These results further confirm our detection of pregnant females during the cold season.

4.4. Diving physiology and pregnancy

We found significant changes in blood and muscle oxygen stores between seasons. Pregnancy can have important effects in the distribution of oxygen stores. Pregnant females face a higher oxygen demand to supply the fetus especially under hypoxic conditions when diving and blood volume typically increases in pregnant mammals. Maternal perfusion of the placenta continues during diving in Weddell seals (Elsner et al., 1970; Liggings et al., 1980).

CSLs exhibited higher levels of Pv and Bv during the warm season (late July) when they have recently given birth (mid-late June, Luque and Auriolos-Gamboa, 2001) and are not pregnant compared to the cold season (late February) when they are in the active gestational phase of pregnancy (~5–6 months, assuming implantation in late Sept). This is contrary to previous studies in humans and other mammals where the plasma volume increases up to 50% during healthy pregnancies (Reynolds, 1953; Bond, 1958; Prince, 1982; Longo, 1983; Hytten, 1985; Letsky, 1995). However, in these studies pregnant and non-pregnant females were not lactating, differing to sea lions in this study. In several terrestrial mammals estrus and ovulation are usually suspended during lactation (lactational anestrus) (Hafez and Hafez, 2000) and therefore they are rarely pregnant while lactating. While we know that lactating female Steller sea lions often conceive during lactation (Atkinson, 1997; Maniscalco et al., 2002, 2006), we do not know the changes, if any, associated with Pv or Bv.

Otariids are unique in that most of the time they are simultaneously pregnant and lactating. Provisioning for the young represents the highest energetic demand during the annual cycle for female California sea lions (Williams et al., 2007). This cost added to pregnancy costs involve great physiological demands that are currently not well understood. After giving birth, there is only a short period when otariids are neither pregnant (2 weeks to 1 month for most otariids (Boyd, 1991b)) nor lactating (if weaning occurs some time before they give birth). Some otariids provision their young until the new pup is born (Trillmich, 1986) and therefore are always lactating (except in failed pregnancies).

High Bvs are maintained throughout lactation in cows, pigs, rabbits and rats (Reynolds, 1953; Bond, 1958; Anderson et al., 1970; Ota and Peaker, 1979) and return to control levels with the cessation of lactation (Reynolds, 1953). In women, Bv values are stabilized after 30 days

postpartum (Caton et al., 1949; Landesman and Miller, 1963). Pv in pigs continues to rise during the first 14 days of lactation and then decreases steadily until weaning. This rise in Pv during the first days of lactation is either to maintain blood flow to the mammary glands or secondary to a general rise in body water (Anderson et al., 1970). These patterns can explain the higher Bv found in CSL females during the warm season when they have recently given birth (~1 month earlier). A rise in body water by means of a rise in Bv during lactation might also be necessary for sea lions to meet milk production requirements. Unlike terrestrial mammals, marine mammals do not have fresh water access and water is obtained mainly from their prey.

Bv in sea lions might start to decrease with the onset of weaning and lactation cessation (such as in the above studies), when sea lion pups are starting to feed on their own. This would explain the lower Bv values found in this study during the cold season (late Feb) when pups are approximately 8 months old. Pups might start to supplement their milk intake by feeding independently at 6 months of age (Melin et al., 2000; Williams et al., 2007). Bv possibly starts to rise again when full lactation resumes after giving birth, corresponding to what we found during the warm season (late July). Similar to results from this study, Galapagos sea lion Bv was also lower in the season when most females were pregnant (Villegas-Amtmann and Costa, 2010). Pregnant northern elephant seals did not show an increase in Bv (Hassrick et al., 2010).

Furthermore, the observed decrease in Bv could be an artifact because the Evan's blue dye does not cross the placenta (Reynolds, 1953; Whipple et al., 1955). Therefore, our Bv measurements only represent the mother and do not include the Bv of the fetus. Since our Bv measurements were determined relative to the total mass of the mother, which includes the fetus, the total Bv for that mass (female and fetus) would be underestimated. In an attempt to correct for this we subtracted 5 kg (estimated fetal mass; J.B.W. Wolf, pers. comm.) from the females' mass and Bv values remained significantly lower during the cold season. Moreover, females gain body weight during pregnancy in addition to fetal mass which would also affect mass specific Bv calculations, but female's mass was not significantly different between seasons.

In contrast to Bv, [Mb] was significantly greater during the cold season, when most females were pregnant. For pregnant sea lions, diving likely results in greater muscular exertion and oxygen consumption than for non-pregnant ones. Noren et al. (2011) found an increase in drag forces, reduction in stroke amplitude and distance per stroke and reductions in swim speeds in pregnant dolphins compared to non-pregnant ones, providing a mechanism for diminished locomotor performance.

Additionally, a dive response (apnea, bradycardia and peripheral vasoconstriction (Irving, 1934; Scholander, 1940; Elsner, 1965; Andersen, 1966)) is necessary to maximize and extend aerobic dive limit. This response should be less pronounced as the level of muscular exertion increases (Davis et al., 2004). Endogenous muscle oxygen stores can only be accessed by the muscle. Therefore, higher [Mb] observed during the cold season could be the result of an increased drag and diminished locomotor performance to be able to maintain the dive response by decreasing muscular exertion and maximize the aerobic dive limit.

We attribute the observed seasonal changes in oxygen stores partly to differences in diving behavior, environmental temperature, and most importantly to reproductive state. The same seasonal pattern in oxygen stores was observed in Galapagos sea lions. They exhibited significantly greater blood oxygen stores and significantly lower muscle oxygen stores during a warm season, compared to a cold one when most females were in a late pregnancy status (Villegas-Amtmann et al., 2009; Villegas-Amtmann and Costa, 2010) further supporting our conclusions.

We found seasonal changes in muscle and blood oxygen stores, but no change in total oxygen stores. Therefore, we suggest that oxygen store studies, in particular those investigating changes or inter-species comparisons, should consider the different oxygen store components

independently as well as consider the blood volume and reproductive state of the animals. By only looking at total oxygen stores one might draw misleading conclusions if the separate components are overlooked.

5. Conclusions

CSL oxygen stores changed between seasons; during the warm season blood oxygen stores were significantly greater and muscle oxygen stores were significantly lower compared to the cold season. Total oxygen stores were not significantly different between seasons.

Although the total oxygen storage capacity remained constant between seasons, blood oxygen stores were greater during the warm and less productive season concurring with sea lions diving deeper and longer (Villegas-Amtmann et al., 2011). Therefore physiological changes in the distribution of oxygen stores can be partly attributed to diving behavior changes driven by prey distribution shifts due to seasonal change (water temperature).

Mean SST was significantly greater during the warm season than during the cold one.

CSL body temperature as approximated by stomach temperature measurements was not significantly different between seasons, but its variance was greater during the warm season.

An increase in oxygen stores during the warm season could be partly attributable to an increase in metabolic rate and therefore oxygen consumption to thermoregulate. Although we did not find changes in total oxygen stores, blood oxygen stores were significantly higher during the warm season. Therefore we partially attribute the observed changes in blood oxygen stores to environmental conditions (ambient and SST).

At least 8 out of 11 females were pregnant during the cold season in February. Pregnancy can have important effects in the distribution of oxygen stores. CSLs exhibited higher levels of Pv and Bv during the warm season when post parturition and during the ovulation and the initial phases of the subsequent pregnancy compared to the cold season when they are in the active gestational phase, approximately 5–6 months pregnant. This differs from previous studies in humans and other mammals. Otariids are unique in that most of the time they are simultaneously pregnant and lactating. In some mammals high Bv is maintained throughout lactation, this can explain the higher Bv found in CSL females during the warm season when they have just given birth about 1 month earlier.

We attribute the observed physiological changes partly to differences in diving behavior, environmental temperature, and most importantly to reproductive state, as the same seasonal pattern in dive physiology was observed in Galapagos sea lions in relation to pregnancy (Villegas-Amtmann and Costa, 2010), further supporting our conclusions.

Blood oxygen stores appear to be more likely influenced by diving behavior and environmental temperature than muscle oxygen stores. Both blood and muscle oxygen stores are influenced by the reproductive state.

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References

- Andersen, H.T., 1966. Physiological adaptations in diving vertebrates. *Physiol. Rev.* 46, 212–243.
- Anderson, D.M., Easley, F.W., McDonald, I., 1970. Blood volume changes during pregnancy and lactation of sows. *Q. J. Exp. Physiol. Cogn. Med. Sci.* 55, 293–300.
- Arnould, J.P.Y., 1995. Indexes of body condition and body-composition in female Antarctic fur seals (*Arctocephalus gazella*). *Mar. Mamm. Sci.* 11, 301–313.
- Atkinson, S., 1997. Reproductive biology of seals. *Rev. Reprod.* 2, 175–194.
- Aurioles, D., Trillmich, F., 2008. *Zalophus californianus*. IUCN: 2008. IUCN Red List of Threatened Species. www.iucnredlist.org.
- Bass, D.E., Kleeman, C.R., Quinn, M., Henschel, A., Hegnauer, A.H., 1955. Mechanisms of acclimatization to heat in man. *Medicine (Baltimore)* 34, 323–380.
- Bond, C.F., 1958. Blood volume changes in the lactating rat. *Endocrinology* 63, 285–289.
- Boyd, I.L., 1991a. Changes in plasma progesterone and prolactin concentrations during the annual cycle and the role of prolactin in the maintenance of lactation and luteal development in the Antarctic fur-seal (*Arctocephalus gazella*). *J. Reprod. Fertil.* 91, 637–647.
- Boyd, I.L., 1991b. Environmental and physiological factors controlling the reproductive-cycles of pinnipeds. *Can. J. Zool./Rev. Can. Zool.* 69, 1135–1148.
- Burns, J.M., Costa, D.P., Frost, K., Harvey, J.T., 2005. Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. *Physiol. Biochem. Zool.* 78, 1057–1068.
- Caton, W.L., Roby, C.C., et al., 1949. Plasma volume and extravascular fluid volume during pregnancy and the puerperium. *Am. J. Obstet. Gynecol.* 57, 471–481.
- Cook, T.R., Lescroel, A., Tremblay, Y., Bost, C.A., 2008. To breathe or not to breathe? Optimal breathing, aerobic dive limit and oxygen stores in deep-diving blue-eyed shags. *Anim. Behav.* 76, 565–576.
- Costa, D.P., Gales, N.J., Crocker, D.E., 1998. Blood volume and diving ability of the New Zealand sea lion, *Phocarctos hookeri*. *Physiol. Zool.* 71, 208–213.
- Costa, D.P., Gales, N.J., Goebel, M.E., 2001. Aerobic dive limit: how often does it occur in nature? *Comp. Biochem. Physiol. A* 129A, 771–783.
- Costa, D.P., Kuhn, C.E., Weise, M.J., Shaffer, S.A., Arnould, J.P.Y., 2004. When does physiology limit the foraging behavior of freely diving mammals? *Int. Congr. Ser.* 1275, 359–366.
- Davis, R.W., Polasek, L., Watson, R., Fuson, A., Williams, T.M., Kanatous, S.B., 2004. The diving paradox: new insights into the role of the dive response in air-breathing vertebrates. *Comp. Biochem. Physiol. A* 138, 263–268.
- El-Sayed, H., Goodall, S.R., Hainsworth, R., 1995. Re-evaluation of Evans blue dye dilution method of plasma volume measurement. *Clin. Lab. Haematol.* 17, 189–194.
- Elsner, R., 1965. Heart Rate Response in Forced Versus Trained Experimental Dives in Pinnipeds. *Hvalradets Skrifter*, 48. Det Norske Videnskaps-Akademi i Oslo, pp. 24–29.
- Elsner, R., Hammond, D.D., Parker, H.R., 1970. Circulatory responses to asphyxia in pregnant and fetal animals: a comparative study of Weddell seals and sheep. *Yale J. Biol. Med.* 42, 202–217.
- Foldager, N., Blomqvist, C.G., 1991. Repeated plasma-volume determination with the Evans blue-dye dilution technique – the method and a computer-program. *Comput. Biol. Med.* 21, 35–41.
- Fowler, S.L., Costa, D.P., Arnould, J.P.Y., Gales, N.J., Burns, J.M., 2007. Ontogeny of oxygen stores and physiological diving capability in Australian sea lions. *Funct. Ecol.* 21, 922–935.
- Gales, N.J., Mattlin, R.H., 1997. Summer diving behavior of lactating New Zealand sea lions, *Phocarctos hookeri*. *Can. J. Zool.* 75, 1695–1706.
- Garcia-Rodriguez, F.J., Aurioles-Gamboa, D., 2004. Spatial and temporal variation in the diet of the California sea lion (*Zalophus californianus*) in the Gulf of California, Mexico. *Fish. Bull.* 102, 47–62.
- Greig, D.J., Mashburn, K.L., Rutishauser, M., Gulland, F.M.D., Williams, T.M., Atkinson, S., 2007. Seasonal changes in circulating progesterone and estrogen concentrations in the California sea lion (*Zalophus californianus*). *J. Mammal.* 88, 67–72.
- Hafez, E.S.E., Hafez, B., 2000. *Reproduction in Farm Animals* 7th edition ed. Lippincott Williams & Wilkins, Baltimore, Maryland, USA.
- Hassrick, J.L., Crocker, D.E., Teutschel, N.M., McDonald, B.I., Robinson, P.W., Simmons, S.E., Costa, D.P., 2010. Condition and mass impact oxygen stores and dive duration in adult female northern elephant seals. *J. Exp. Biol.* 213, 585–592.
- Hedrick, M.S., Duffield, D.A., 1991. Hematological and rheological characteristics of blood in 7 marine mammal species – physiological implications for diving behavior. *J. Zool.* 225, 273–283.
- Horning, M., Trillmich, F., 1997. Development of hemoglobin, hematocrit, and erythrocyte values in Galapagos fur seals. *Mar. Mamm. Sci.* 13, 100–113.
- Houston, A.H., Gingrasbedard, J.H., 1994. Variable versus constant-temperature acclimation regimes – effects on hemoglobin isomorph profile in goldfish, *Carassius auratus*. *Fish. Physiol. Biochem.* 13, 445–450.
- Hytten, F., 1985. Blood-volume changes in normal-pregnancy. *Clin. Haematol.* 14, 601–612.
- Irving, L., 1934. On the ability of warm-blooded animals to survive without breathing. *Sci. Mon.* 38, 422–428.
- Kodama, A.M., Elsner, R., Pace, N., 1977. Effects of growth, diving history, and high altitude on blood oxygen capacity in harbor seals. *J. Appl. Physiol.* 42, 852–858.
- Kooyman, G.L., 1973. Respiratory adaptations in marine mammals. *Am. Zool.* 13, 457–468.

- Kooyman, G.L., 1989. *Diverse Divers: Physiology and Behavior*. Springer-Verlag, Berlin Heidelberg.
- Kooyman, C.A., P.J., P., 1998. *The Physiological Basis of Diving to Depth: Birds and Mammals*. Annual Reviews Inc., Palo Alto, California, USA.
- Kooyman, G.L., Ponganis, P.J., 1990. *Behavior and Physiology of Diving in Emperor and King Penguins*. Academic Press, New York.
- Kooyman, G.L., Sinnett, E.E., 1982. Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. *Physiol. Zool.* 55, 105–111.
- Kooyman, G.L., Castellini, M.A., Davis, R.W., Maue, R.A., 1983. Aerobic diving limits of immature Weddell seals. *J. Comp. Physiol.* 151, 171–174.
- Kotiaho, J.S., 1999. Estimating fitness: comparison of body condition indices revisited. *Oikos* 87, 399–400.
- Lamb, A., Edgell, P., 1986. *Coastal Fishes of the Pacific Northwest*. Harbour Publishing Co. Ltd., British Columbia, Canada.
- Landesman, R., Miller, M.M., 1963. Blood volume changes during the immediate post-partum period. *Obstet. Gynecol.* 21, 40–48.
- Lenfant, C., Johansen, K., Torrance, J.D., 1970. Gas transport and oxygen storage capacity in some pinnipeds and sea otter. *Respir. Physiol.* 9, 277.
- Letsky, E.A., 1995. Erythropoiesis in pregnancy. *J. Perinat. Med.* 23, 39–45.
- Liao, J.A., 1990. An Investigation of the Effect of Water Temperature on the Metabolic Rate of the California Sea Lion (*Zalophus californianus*). : Marine Sciences. University of California Santa Cruz, Santa Cruz, p. 55.
- Liggings, G.C., Qvist, J., Hochachka, P.W., Murphy, B.J., Creasy, R.K., Schneider, R.C., Snider, M.T., Zapol, W.M., 1980. Fetal cardiovascular and metabolic responses to simulated diving in the Weddell seal. *J. Appl. Physiol.* 49, 424–430.
- Longo, L.D., 1983. Maternal blood-volume and cardiac-output during pregnancy – a hypothesis of endocrinologic control. *Am. J. Physiol.* 245, R720–R729.
- Luque, S.P., Aurioules-Gamboia, D., 2001. Sex differences in body size and body condition of California sea lion (*Zalophus californianus*) pups from the Gulf of California. *Mar. Mamm. Sci.* 17, 147–160.
- Lutcavage, M.E., Bushnell, P.G., Jones, D.R., 1992. Oxygen stores and aerobic metabolism in the leatherback sea-turtle. *Can. J. Zool.* 70, 348–351.
- MacArthur, R.A., Wesen, G.L., Campbell, K.L., 2003. Diving experience and the aerobic dive capacity of muskrats: does training produce a better diver? *J. Exp. Biol.* 206, 1153–1161.
- Maniscalco, J.M., Atkinson, S., Armato, P., 2002. Early maternal care and pup survival in Steller sea lions: a remote video monitoring project in the northern Gulf of Alaska. *Arctic Res. U. S.* 16, 36–41.
- Maniscalco, J.M., Wynne, K., Pitcher, K.W., Hanson, M.B., Melin, S.R., Atkinson, S., 2004. The occurrence of California sea lions (*Zalophus californianus*) in Alaska. *Aquat. Mamm.* 30, 427–433.
- Maniscalco, J.M., Harris, K.R., Atkinson, S., Parker, P., 2006. Alloparenting in Steller sea lions (*Eumetopias jubatus*): correlations with misdirected care and other observations. *J. Ethol.* 25, 125–131.
- Mate, B.R., Lagerquist, B.A., Calambokidis, J., 1999. Movements of North Pacific blue whales during the feeding season off southern California and their southern fall migration. *Mar. Mamm. Sci.* 15, 1246–1257.
- Matsuura, D.T., Whittow, G.C., 1973. Oxygen-uptake of California sea lion and harbor seal during exposure to heat. *Am. J. Physiol.* 225, 711–715.
- McIntyre, I.W., Campbell, K.L., MacArthur, R.A., 2002. Body oxygen stores, aerobic dive limits and diving behaviour of the star-nosed mole (*Condylura cristata*) and comparisons with non-aquatic talpids. *J. Exp. Biol.* 205, 45–54.
- Meir, J.U., Champagne, C.D., Costa, D.P., Williams, C.L., Ponganis, P.J., 2009. Extreme hypoxic tolerance and blood oxygen depletion in diving elephant seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R927–R939.
- Melin, S.R., DeLong, R.L., Thomason, J.R., VanBlaricom, G.R., 2000. Attendance patterns of California sea lion (*Zalophus californianus*) females and pups during the non-breeding season at San Miguel Island. *Mar. Mamm. Sci.* 16, 169–185.
- Noren, S.R., Lacave, G., Wells, R.S., Williams, T.M., 2002. The development of blood oxygen stores in bottlenose dolphins (*Tursiops truncatus*): implications for diving capacity. *J. Zool.* 258, 105–113.
- Noren, S.R., Redfern, J.V., Edwards, E.F., 2011. Pregnancy is a drag: hydrodynamics, kinematics and performance in pre- and post-parturition bottlenose dolphins (*Tursiops truncatus*). *J. Exp. Biol.* 214, 4151–4159.
- Odell, D.K., 1975. Breeding biology of the California sea lion (*Zalophus californianus*). *Rapp. P.V. Reun. Cons. Int. Explor. Mer.* 169, 374–378.
- Ota, K., Peaker, M., 1979. Lactation in the rabbit: mammary blood flow and cardiac output. *Q. J. Exp. Physiol. Cogn. Med. Sci.* 64, 225–238.
- Parás, A., 2008. *Capture and Anesthesia of Otariids in the Wild*. Saunders Elsevier, St. Louis, Missouri.
- Ponganis, P.J., 2011. Diving mammals, comprehensive physiology. *Am. Physiol. Soc.* 447–465.
- Ponganis, P.J., Kooyman, G.L., Sartoris, D., Jobsis, P., 1992. Pinniped splenic volumes. *Am. J. Physiol.* 262, R322–R325.
- Ponganis, P.J., Kooyman, G.L., Castellini, M.A., 1993. Determinants of the aerobic dive limit of Weddell seals – analysis of diving metabolic rates, postdive end-tidal P_{O_2} , and blood and muscle oxygen stores. *Physiol. Zool.* 66, 732–749.
- Ponganis, P.J., Kooyman, G.L., Winter, L.M., Starke, L.N., 1997. Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions, *Zalophus californianus*. *J. Comp. Physiol. B* 167, 9–16.
- Prince, H., 1982. Blood-volume in the pregnant rabbit. *Q. J. Exp. Physiol. Cogn. Med. Sci.* 67, 87–95.
- Reijnders, P.J.H., 1990. Progesterone and estradiol-17-beta concentration profiles throughout the reproductive-cycle in harbor seals (*Phoca vitulina*). *J. Reprod. Fertil.* 90, 403–409.
- Reynafarje, B., 1963. Simplified method for determination of myoglobin. *J. Lab. Clin. Med.* 61, 138.
- Reynolds, M., 1953. Measurement of bovine plasma and blood volume during pregnancy and lactation. *Am. J. Physiol.* 175, 118–122.
- Richmond, J.P., Burns, J.M., Rea, L.D., 2006. Ontogeny of total body oxygen stores and aerobic dive potential in Steller sea lions (*Eumetopias jubatus*). *J. Comp. Physiol. B* 176, 535–545.
- Riede, K., 2004. Global register of migratory species – from global to regional scales. Final Report on the R&D – Projekt 808 05 081. Federal Agency for Nature Conservation, Bonn, Germany, p. 329.
- Scholander, P.F., 1940. Experimental investigations on the respiratory function in diving birds and mammals. *Hvalradets Skrifter* 22, 1–131.
- Schooley, R.L., McLaughlin, C.R., Matula, G.J., Krohn, W.B., 1994. Denning chronology of female black bears – effects of food, weather, and reproduction. *J. Mammal.* 75, 466–477.
- Senay, L.C., Kok, R., 1976. Body fluid responses of heat-tolerant and intolerant men to work in a hot wet environment. *J. Appl. Physiol.* 40, 55–59.
- Shapiro, Y., Hubbard, R.W., Kimbrough, C.M., Pandolf, K.B., 1981. Physiological and hematologic responses to summer and winter dry-heat acclimation. *J. Appl. Physiol.* 50, 792–798.
- Stephenson, R., Turner, D.L., Butler, P.J., 1989. The Relationship between diving activity and oxygen storage capacity in the tufted duck (*Aythya fuligula*). *J. Exp. Biol.* 141, 265–275.
- Swan, H., Nelson, A.W., 1971. Blood-volume measurement – concepts and technology. *J. Cardiovasc. Surg.* 12, 389.
- Szteren, D., Aurioules, D., Gerber, L., 2006. Population status and trends of the California sea lion in the Gulf of California, Mexico. In: Trites, A., Atkinson, S., DeMaster, D.P., Fritz, L.W., Gelatt, T.S., Re, L., Wynne, K. (Eds.), *Sea Lions of the World*. University of Alaska Fairbanks, Alaska, pp. 369–384.
- Tanaka, H., 2006. Winter hibernation and body temperature fluctuation in the Japanese badger, *Meles meles anakuma*. *Zool. Sci.* 23, 991–997.
- Tracy, C.R., Reynolds, S.J., McArthur, L., Tracy, C.R., Christian, K.A., 2007. Ecology of aestivation in a cocoon-forming frog, *Cyclorana australis* (Hylidae). *Copeia* 901–912.
- Tremblay, Y., Shaffer, S.A., Fowler, S.L., Kuhn, C.E., McDonald, B.I., Weise, M.J., Bost, C.A., Weimerskirch, H., Crocker, D.E., Goebel, M.E., Costa, D.R., 2006. Interpolation of animal tracking data in a fluid environment. *J. Exp. Biol.* 209, 128–140.
- Trillmich, F., 1986. Attendance Behavior of Galapagos Sea Lions in Fur seals: Maternal Strategies on Land and Sea. Princeton University Press.
- Urquhart, F.A., Urquhart, N.R., 1978. Autumnal migration routes of eastern population of monarch butterfly (*Danaus p-plexippus* L-Danaidae-Lepidoptera) in North-America to overwintering site in Neovolcanic Plateau of Mexico. *Can. J. Zool.* 56, 1759–1764.
- Villegas-Amtmann, S., Costa, D.P., 2010. Oxygen stores plasticity linked to foraging behaviour and pregnancy in a diving predator, the Galapagos sea lion. *Funct. Ecol.* 24, 785–795.
- Villegas-Amtmann, S., Atkinson, S., Costa, D.P., 2009. Low synchrony in the breeding cycle of Galapagos sea lions revealed by seasonal progesterone concentrations. *J. Mammal.* 90, 1232–1237.
- Villegas-Amtmann, S., Simmons, S.E., Kuhn, C.E., Huckstadt, L.A., Costa, D.P., 2011. Latitudinal range influences the seasonal variation in the foraging behavior of marine top predators. *PLoS One* 6 (8), e23166. <http://dx.doi.org/10.1371/journal.pone.0023166>.
- Weise, M.J., Costa, D.P., 2007. Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. *J. Exp. Biol.* 210, 278–289.
- Whipple, G.H., Hill, R.B., Terry, R., Lucas, F.V., Yuile, C.L., 1955. The placenta and protein metabolism – transfer studies using carbon-14-labeled proteins in dogs. *J. Exp. Med.* 101, 617–626.
- Williams, T.M., Rutishauser, M., Long, B., Fink, T., Gafney, J., Mostman-Liwanag, H., Casper, D., 2007. Seasonal variability in otariid energetics: implications for the effects of predators on localized prey resources. *Physiol. Biochem. Zool.* 80, 433–443.
- Wyndham, C.H., Benade, A.J., Williams, C.G., Strydom, N.B., Goldin, A., Heyns, A.J., 1968. Changes in central circulation and body fluid spaces during acclimatization to heat. *J. Appl. Physiol.* 25, 586–593.