

Oxygen stores plasticity linked to foraging behaviour and pregnancy in a diving predator, the Galapagos sea lion

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Summary

1. Survival of a population can be influenced by foraging success that is determined by ecological and physiological factors. Foraging in air-breathing marine vertebrates is constrained by the duration of their dives, which is in part determined by their oxygen stores. Such organisms are excellent models to study how and when physiology limits behaviour and the associated role of ecological factors such as seasonal change.

2. The Galapagos sea lion (GSL) *Zalophus wollebaeki* is endangered and its population is declining. Previous work found that GSL exhibited three distinct foraging strategies during a warm season when resources are less abundant, possibly caused by intraspecific competition.

3. We examined adult female GSL diving behaviour during a cold, productive season compared to a warm season to test the hypothesis that they would converge onto one foraging strategy as a consequence of increased productivity. We investigated intra and inter-seasonal changes in GSL oxygen stores during a warm (March 2005, $n = 11$) and cold season (August–September 2006, $n = 12$) at Caamaño Islet, Galapagos.

4. GSL exhibited persistence of three foraging strategies during the cold, productive season similar to those found during the warm season. This suggests that intraspecific competition associated with limited resources in this area of the Galapagos Islands is maintained throughout the year.

5. GSL exhibited contrasting seasonal changes in their oxygen stores. During the warm season haemoglobin (Hb) and blood volume (Bv) were significantly higher while myoglobin (Mb) was significantly lower compared to that in the cold season. Given that diving behaviour was similar between seasons, these changes are attributed to other causes such as pregnancy as most females were pregnant during the cold season.

6. GSL exhibited physiological plasticity within each season that was attributed to dive behaviour. Oxygen stores of the three foraging strategies, increased according to dive durations: haematocrit, Hb and Mb during the warm season and Hb, Mb and Bv during the cold season.

7. Knowledge of an organism's physiological plasticity is important to assess its vulnerability to climate change. We document for the first time inter and intraseasonal physiological response coupled with diving behaviour in the same sex and age class of an air-breathing diving vertebrate.

Key-words: diving behaviour, diving physiology, foraging strategies, Galapagos Islands, physiological plasticity, pinnipeds, seasonal diving behaviour, *Zalophus wollebaeki*

Introduction

Foraging success, influenced by ecological and physiological factors, is a key factor in the persistence of many populations. Ecological factors include distribution, abundance

and energy content of prey, as well as abiotic factors such as environmental temperature. Physiological factors consist of an organism's performance capability (Swallow *et al.* 2009). Air-breathing marine vertebrates that have secondarily returned to the ocean are an excellent model to study how physiological constraint interacts with ecological factors. These diverse marine vertebrates include sea snakes, sea

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turtles, the marine iguana, marine crocodiles, pinnipeds, cetaceans, sea otters, manatees and dugongs. Regardless of their taxonomic origin, all of these animals face the same fundamental constraint, a limited amount of oxygen that constrains the duration of their foraging dives while foraging (Costa 2007). The primary physiological factors that determine the diving capability of an air-breathing marine vertebrate are its oxygen stores and the rate at which they are used (Kooyman 1989). Oxygen stores are thought to be the major determinant of diving performance (Kooyman *et al.* 1980, 1983; Kooyman 1989; Ponganis, Kooyman & Castellini 1993; Ponganis *et al.* 1997). The role of behaviour in the physiology of diving mammals is shown in their higher oxygen storage capacity in blood and muscle compared to terrestrial mammals of similar size (Kooyman 1989). Oxygen storage capacity also varies across this group of diving mammals according to their dive durations as observed in different pinniped species (seals, sea lions and fur seals) (Costa *et al.* 2004).

The behaviour and physiology of an animal are also affected by environmental conditions, such as ambient temperature. Furthermore, seasonal changes in marine ecosystems influence the distribution and availability of prey for predatory vertebrates, which in turn is often reflected by changes in their diving behaviour. In general, seasonal differences in the behaviour and physiology of marine tetrapods might be expected to be greater in species living at high latitude compared with those living closer to the equator.

Diving behaviour have been described for many air-breathing diving vertebrates (Halsey, Blackburn & Butler 2006), including all extant sea lion species (Shaffer & Costa 2006). Seasonal variation in diving behaviour has only been examined in a few of these studies and includes for example penguins (Kirkwood & Robertson 1997), seals (Bennett, McConnell & Fedak 2001; Burns & Kooyman 2001; Burns *et al.* 2004) and sea lions (Merrick & Loughlin 1997; Costa & Gales 2003). These studies showed pronounced seasonal variation in diving behaviour. While some studies have reported seasonal differences in diving behaviour no study has examined seasonal changes in the physiological capability in the same age and size class. Studying seasonal physiological capabilities of these diving mammals is essential to determine if and how their physiology tracks behaviour.

The Galapagos Archipelago is a dynamic ecosystem characterized by two contrasting seasons, a cool and dry season (May–December) and a warm and wet season (December–May). Sea surface and ambient temperature varies *c.* 10–12 °C between seasons (<http://coastwatch.pfeg.noaa.gov/>). The warm season is characterized by lower upwelling, warmer water temperatures (~25 °C), reduced productivity and lower prey abundance (Pak & Zanveld 1973; Feldman 1986). Sea lions living in ecological and thermally variable environments require behavioural and physiological adaptations to thermoregulate and stay in energy balance. Seasonal prey abundance and distribution changes in the Galapagos Archipelago may also influence sea lions' diving behaviour while water temperature changes may influence its physiological response.



Fig. 1. Galapagos sea lion, *Zalophus wollebaeki*.

Seasonal diving behaviour in same age class individuals has never been studied in a low latitude marine mammal or sea-bird such as the Galapagos sea lion (GSL) (*Zalophus wollebaeki*, Sivertsen, 1953) (Fig. 1) and its diving physiology and oxygen storage capacity have never been determined. The GSL is an endemic and endangered species (Aurioles & Trillmich 2008) found in the Galapagos Archipelago whose population is declining (Heath 2002) and is negatively affected by El Niño events (Trillmich & Limberger 1985). The GSL is an important vector for the transport of marine nutrients to the terrestrial ecosystem (Fariña *et al.* 2003). Therefore, knowledge about this species is vital not only for its own conservation but also for the health of the ecosystem in which it lives. The objectives of this study were to determine if the GSL diving behaviour and physiology change in response to changing seasonal and environmental conditions. During the warm and least productive season GSL diving behaviour exhibited three distinct foraging patterns, possibly due to increased intraspecific competition for food (Villegas-Amtmann *et al.* 2008). In this study, we hypothesize: (i) that GSL would exhibit a change in their diving behaviour during the cold and productive season, converging on one foraging strategy as a consequence of increased availability of resources and therefore reduced competition; (ii) that there would be a change in their oxygen stores associated with changes in behaviour related to seasonal or environmental changes (temperature and or productivity).

Materials and methods

FIELD SITE AND TAGGING PROCEDURES

Research was carried out during two contrasting seasons: a warm season (March 2005) and a cold one (August–September 2006) at Camaño Islet (0°759'S, 90°278°W) in the Galapagos Islands. During the warm season we captured 11 lactating female GSL, which were suckling small pups, 4–5 months after peak pupping season. During the cold season we captured 12 lactating female GSL, most of them with pups that were bigger and possibly older (10–11 months after peak pupping season) than the ones from the previous season. Of these five

of 11 females during the warm season and nine of 12 females during the cold season were pregnant (Villegas-Amtmann, Atkinson & Costa 2009). Sea lions were captured with hoop nets and anaesthetized with isoflurane gas (0.5–2.5%) with oxygen via a portable field vaporizer, administered initially through a cone-shaped mask and afterwards with an endotracheal tube (Gales & Mattlin 1997). Once under anaesthesia we took muscle biopsies, blood samples and instrumented the sea lions.

To determine movement patterns at sea we instrumented nine of the 11 animals with SPOT4 satellite platform terminal transmitters (PTT) during the warm season (2005), and a total of 12 animals during the cold season (2006), seven with SPOT5 PTTs and five with Mk10-AF (Wildlife Computers, Richmond, WA, USA). Diving behaviour data were obtained by instrumenting sea lions with time-depth recorders (TDR), two Mk6, two Mk8 and five Mk9 models in 2005 and seven Mk9 and five Mk10-AF in 2006 (Wildlife Computers). Animals were instrumented with radio transmitters (VHF; Sirtrack, Havelock, North, New Zealand) to aid in recovery on land. We mounted instruments on mesh netting and glued them to the dorsal pelage of the lower back and between the shoulders of the animals using 5 min quick set Loctite epoxy. The total weight of the instruments attached was *c.* 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. We recaptured all the sea lions after 7–11 days during the warm season (2005), and all but one female (CAAF06) after 14–21 days during the cold season (2006). The equipment was removed by physically restraining the animals without anaesthesia. Epoxy mounts fall off within a few months during the animals' annual moult.

PHYSIOLOGICAL SAMPLE COLLECTION AND ANALYSIS

We measured the sea lions' oxygen stores by collecting blood and muscle samples. We collected blood samples from the caudal-gluteal vein for haematocrit (Hct) and haemoglobin (Hb) determination from all females in both seasons. Blood samples used to determine Hct were taken before anaesthesia induction because Hct declines under general anaesthesia (Ponganis *et al.* 1992; Costa, Gales & Crocker 1998). Hb concentration was measured following the cyanmethaemoglobin photometric method (Stanbio Laboratory, Boerne, TX, USA). This was done by pipetting 10 μ L of whole blood into 2.5 mL of Drabkin's reagent (Ricca Chemical Company, Arlington, TX, USA) in the field, the samples were then stored in the dark for later analysis in the laboratory. We calculated mean corpuscular haemoglobin content (MCHC) using the equation: $MCHC = Hb/Hct$.

To determine plasma (Pv) and blood (Bv) volumes, we injected an intravenous dose of *c.* 0.6 mg kg⁻¹ of Evan's blue dye (Sigma Diagnostics, St. Louis, MO, USA) into the caudal-gluteal or jugular vein followed by the collection of three blood samples at 10, 20 and 30 min after the injection. Injections and samples were given and taken through needles. The amount of dye injected was determined by weighing the injectate contained in syringe with a portable balance accurate to 0.01 g. Only injections where the injectate was completely introduced into the vein were used. This was determined by the ability to flush the injection syringe three times with blood after introduction of the dye. Data were not used from four animals where it was unclear that all of the injectate had been completely introduced. Pv and Bv were determined after storage by photometric absorbance of the centrifuged plasma samples at 624 and 740 nm and the calculation of the dye concentration at the time of injection following the Evan's blue method (Swan & Nelson 1971; Foldager & Blomqvist 1991; El-Sayed, Goodall & Hainsworth 1995; Costa, Gales & Crocker 1998).

To determine muscle oxygen stores we used a 6 mm dermal biopsy punch (Miltex, Inc., York, PA, USA) to obtain a muscle biopsy from a primary locomotor muscle, supraspinatus muscle, above the sea lions' pectoral flipper. Subsequently after storage at -80 °C we obtained myoglobin (Mb) concentration in the muscle following the method of Reynafarje (1963).

We calculated total body oxygen stores by adding blood, muscle and lung oxygen stores (Lenfant, Johansen & Torrance 1970; Kooyman *et al.* 1971, 1983; Kooyman 1989; Ponganis, Kooyman & Castellini 1993; Ponganis *et al.* 1997; Davis & Kanatous 1999; Costa, Gales & Goebel 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 following Costa, Gales & Goebel (2001).

TRACKING AND DIVING BEHAVIOUR ANALYSES

To determine the sea lions' habitat utilization and foraging range we used custom software developed by Y. Tremblay (IKNOS toolbox) to filter Argos location data. The algorithm uses several criteria to remove unlikely locations: (i) realistic travel speeds of a subject between two fixes (≤ 10 km h⁻¹), (ii) change in azimuth between successive fixes, (iii) Argos location class and (iv) time lapse between two consecutive fixes (Tremblay *et al.* 2006). We plotted filtered locations using Matlab (The MathWorks Inc., Natick, MA, USA).

We analysed dive data in Matlab (The MathWorks Inc.) using a custom-written dive analysis program (Y. Tremblay, unpublished data), which allows for a zero offset correction at the surface and the identification of dives based on a minimum depth and duration. Diving data from the warm season (2005) are published (Villegas-Amtmann *et al.* 2008) and diving data from the cold season (2006) are presented here and were analysed following the same methods. All Mk9 and Mk10-AF recorders in the cold season had a 0.5 m depth resolution and were sampled every 2 s. The minimum depth considered for a dive was 5 m and the minimum duration was 12 s by setting a minimum of six sampling points for dive identification by the dive analysis program ($6 \times \text{sampling rate} - 2$ s).

We discarded 'porpoising' or shallow dives that sea lions exhibit when travelling to different locations from our analysis as in Villegas-Amtmann *et al.* (2008). These dives were restricted to the upper 6–8 m. 'Porpoising' dives are the shallowest in the diving record and their recognition is dependent on the recorders' sampling resolution. Therefore, exclusion of these dives from our analyses eliminated dissimilarity effects in sampling resolution between the different recorders used within and between seasons. This was also consistent with our goal of analysing potential foraging dives (i.e. feeding or search dives).

We conducted multivariate analyses (SYSTAT 10.2) to classify diving behaviour as in Villegas-Amtmann *et al.* (2008). Data were tested for normality by visual examination and no transformations were needed. Variables used are female's means calculated from averages of the following for each dive: maximum depth (m), dive duration (s), bottom time (s) (calculated using a depth threshold of 80% of the maximum dive depth), number of 'wiggles' at the bottom of a dive (number of ascent and descent movements at the bottom of the dive, which imply foraging behaviour) (Kuhn 2006), descent and ascent rate (m s⁻¹) and dive rate (dives h⁻¹). Mass (kg) was also included as a variable.

Principal component analysis (PCA) was used to reduce the number of variables, followed by a hierarchical cluster analysis (HCA) to classify diving behaviour. We used Latent root criterion, a minimum eigenvalue of 1.0 and a varimax rotation for the PCA. This analysis is

suitable for this type of data because the diving variables are strongly correlated (McGarigal, Cushman & Stafford 2000). Standardized PCA factor scores were input as variables in the HCA which we conducted using Euclidean distance and average linking method. Hierarchical clustering is ideal for small data sets as in this study (McGarigal, Cushman & Stafford 2000).

To further characterize diving behaviour, we used 'intra-depth zone' measurements (IDZ) (Tremblay & Chérel 2003). IDZ provides an index of the tendency to repeatedly dive to a given depth. Considering 5 m was the minimum detectable depth for a dive, we applied a user defined zone of ± 10 m of the maximum depth of the previous dive (i.e. 5 m above and below the previous depth) to calculate IDZ, evidence of benthic diving. Data are presented as mean ± 1 standard deviation.

Results

DIVING BEHAVIOUR COLD SEASON

During the cold season 11 of 12 females were recaptured. One female's TDR was missing and another female (CAAFC08) exhibited abnormal or barely any diving behaviour, as this animal was consistently seen near a dock where fishermen cleaned their fish. This female was therefore treated as an outlier and not included in the dive behaviour analyses. Diving behaviour data were therefore obtained from 10 GSL during the cold season. TDR deployment duration varied from 12 to 19 days with data collected over a mean of 14.15 ± 2.36 days ($n = 9$). Elimination of porpoising dives resulted in the analysis of 1393 ± 616 (range 319–2380) dives per female, accounting for 94–100% of the total number of dives.

At sea, females spent $53.9 \pm 7.5\%$ of their time diving. Mean individual dive depth ranged from 30 to 193 m and averaged 93 ± 47 m ($n = 9$). Mean individual dive durations ranged from 2.6 to 6.8 min, with an average of 4.4 ± 1.4 min ($n = 9$) (Table 1). As observed during the warm season, animals displayed individual variation in the time of day they dived. Four females dived exclusively during the day, two individuals dived exclusively during the night and the remaining three animals exhibited no diel pattern.

The multivariate analysis revealed three factors from the PCA that explained 88.3% of the variance and therefore were used for the subsequent analysis (HCA). Dive duration and bottom time explained most of the variance in factor 1. Factor 2 was driven by dive rate and factor 3 by mass (Table 2). The HCA identified three groups of animals also found during the warm season (Villegas-Amtmann *et al.* 2008). The maximum Euclidean distance for a group to be considered was 0.94 based on the cluster tree produced by the HCA. The three groups from the cold season presented similar diving behaviour characteristics as the groups found during the warm season. Groups were classified in the same manner with a minor modification in group 2 as follows: (i) shallow divers, (ii) deep bottom divers and (iii) bottom divers (Fig. 2, Table 1).

Shallow divers (CAAFC01, CAAFC04; Table 1) exhibited similar diving characteristics as the warm season shallow divers (shallowest mean dive depth, shortest dive duration

Table 1. *Zatophus wollebaeki*. Mean (\pm SD) and maximum dive parameters. Caamaño Islet, August–September 2006. IDZ (intra-depth zone), similar max. dive depth on consecutive dives

| Dive strategy | Female ID | Mass (kg) | Dive depth (m) | | Dive duration (min) | | Bottom time (min) | | Mean no. bottom wiggles | Mean descent rate ($m s^{-1}$) | Mean ascent rate ($m s^{-1}$) | Mean dive rate (dives h^{-1}) | % IDZ | % Time diving at sea |
|---------------|-----------|-----------|------------------|-------|---------------------|------|-------------------|------|-------------------------|----------------------------------|---------------------------------|----------------------------------|-------|----------------------|
| | | | Mean | Max. | Mean | Max. | Mean | Max. | | | | | | |
| Shallow | CAAFC01 | 80.5 | 30.4 \pm 8.2 | 71 | 3.1 \pm 1 | 7.7 | 2.2 \pm 1 | 7.2 | 13.1 \pm 6.9 | 1.3 \pm 0.4 | 0.9 \pm 0.3 | 12.8 \pm 4.1 | 66.1 | 67.6 |
| Shallow | CAAFC04 | 75.5 | 47.2 \pm 47.3 | 252 | 2.6 \pm 1.8 | 9.4 | 1.1 \pm 1.1 | 6.3 | 5.7 \pm 5.8 | 0.8 \pm 0.4 | 0.1 \pm 0.5 | 13.6 \pm 9.2 | 29.0 | 63.8 |
| Bottom | CAAFC03 | 77.5 | 85.9 \pm 36.8 | 166.5 | 3.8 \pm 1.5 | 9.5 | 1.9 \pm 1 | 5.3 | 9.7 \pm 6.3 | 1.5 \pm 0.5 | 1.3 \pm 0.4 | 8.1 \pm 3.5 | 74.7 | 53.5 |
| Bottom | CAAFC05 | 62 | 83.9 \pm 31.1 | 228 | 4.1 \pm 1 | 7.5 | 2.2 \pm 0.8 | 5 | 10.1 \pm 5.1 | 1.5 \pm 0.4 | 1.4 \pm 0.4 | 8.2 \pm 3.4 | 83.6 | 57.0 |
| Bottom | CAAFC12 | 67 | 80.1 \pm 46.3 | 245 | 4.3 \pm 1.1 | 8.8 | 2.6 \pm 0.8 | 5.2 | 14.2 \pm 5.8 | 1.7 \pm 0.3 | 1.5 \pm 0.3 | 9.2 \pm 3.6 | 86.8 | 58.2 |
| Deep bottom | CAAFC02 | 92 | 127.9 \pm 79.9 | 387 | 6.1 \pm 2.1 | 11.1 | 2.9 \pm 1.5 | 6.4 | 16.6 \pm 9.6 | 1.3 \pm 0.4 | 1.2 \pm 0.4 | 5.6 \pm 2.3 | 68.8 | 57.4 |
| Deep bottom | CAAFC07 | 81.5 | 97.5 \pm 27.6 | 180 | 5.9 \pm 1.5 | 10 | 3.6 \pm 1.3 | 7.2 | 20.3 \pm 9.1 | 1.4 \pm 0.3 | 1.4 \pm 0.3 | 6.4 \pm 1.9 | 89.0 | 63.3 |
| Deep bottom | CAAFC09 | 92 | 88.4 \pm 82.3 | 283 | 3.4 \pm 2.5 | 10.1 | 1.3 \pm 1.2 | 6.2 | 7.6 \pm 6.7 | 1.3 \pm 0.5 | 1.2 \pm 0.6 | 7.4 \pm 6.2 | 48.0 | 41.6 |
| Deep bottom | CAAFC10 | 78 | 192.6 \pm 83.2 | 360 | 6.8 \pm 2.4 | 10.3 | 3.0 \pm 1.3 | 6 | 17.9 \pm 9.7 | 1.6 \pm 0.5 | 1.6 \pm 0.5 | 4.3 \pm 1.8 | 78.9 | 46.3 |

Table 2. *Zalophus wollebaeki*. PCA rotated loading matrix (SYSTAT 11) for dive parameters used in the diving behavior analysis of 10 females from Caamaño Islet during August–September 2006. In bold are loadings for the diving variables that contributed the most for Factors 1, 2 and 3

| Dive parameters | Factor 1 (61.61%) | Factor 2 (13.83%) | Factor 3 (12.86%) |
|-------------------------|----------------------|----------------------|----------------------|
| Mean dive depth | 0.862 | -0.065 | -0.212 |
| Mean dive duration | 0.963 | 0.017 | -0.157 |
| Mean bottom time | 0.918 | 0.069 | 0.096 |
| Mean no. bottom wiggles | 0.899 | 0.037 | -0.03 |
| Mass | 0.348 | 0.532 | -0.712 |
| Mean descent rate | 0.782 | 0.26 | 0.488 |
| Mean ascent rate | 0.858 | -0.152 | 0.304 |
| Mean dive rate | -0.37 | 0.85 | 0.331 |

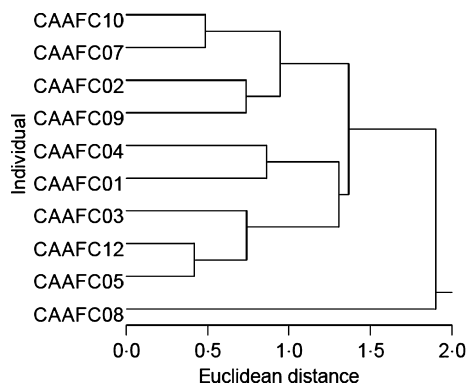


Fig. 2. Cluster tree of dive parameters from 10 female Galapagos sea lions from Caamaño Islet, August–September 2006, showing four distinct groups, with distinct foraging strategies (SYSTAT 10.2). CAAFC08 was an outlier and taken out of further analysis. Euclidean distance considered for clustering was 0.94 based on the unique shared characteristics of each group.

and highest mean dive rate of all females) with the exception that they were not the smallest animals and were day and night divers, compared to smallest animals and night divers during the warm season. They spent on average 65.7% of their time at sea diving.

Deep bottom divers (CAAFC02, CAAFC07, CAAFC09, CAAFC10; Table 1) shared characteristics with the 'deep divers' group found in the warm season (deepest mean dive depths, shortest mean dive rate of all females in the study and predominantly day divers), but did not exhibit the shortest mean bottom time nor the lowest proportion of IDZ dives (similar depths on consecutive dives). This group exhibited the longest bottom time and dive duration of all groups and spent an average of 52.2% of their time at sea diving.

Bottom divers (CAAFC03, CAAFC05, CAAFC12; Table 1) also presented characteristics exhibited during the warm season (highest mean ascent and descent rate, highest proportion of IDZ dives and dived during all times of day). Characteristics not shared during this season were longest mean bottom time and longest mean dive duration (exhibited by the deep bottom divers in the cold season). These animals spent an average of 56.3% of their time at sea diving.

The three diving groups showed similar spatial segregation in their foraging locations obtained from their ARGOS positions as observed during the warm season (Villegas-Amtmann *et al.* 2008; Fig. 3). We compared diving behaviour between seasons by testing data for normality and performing *t*-tests (SYSTAT 10.2).

PHYSIOLOGICAL PLASTICITY AND FORAGING STRATEGIES

We tested data for normality using a Kolmogorov–Smirnov one sample test. We compared blood and muscle oxygen stores between seasons by performing *t*-tests. We performed linear regressions to examine the relationships between dive duration and oxygen stores, and body mass and dive duration for each season.

Three foraging patterns were identified during the warm season, described as 'shallow, deep and bottom divers'. Individuals in these groups exhibited distinct and increasing mean dive durations: 2.94 ± 0 ($n = 2$), 3.85 ± 0.25 ($n = 4$) and 4.94 ± 0.94 ($n = 3$) min respectively (Villegas-Amtmann *et al.* 2008). Mean oxygen stores, Hct, Hb and Mb, of these groups exhibited trends that increased according to dive durations: Hct, 55.5 ± 0.7 , 58 ± 3.7 and $58.7 \pm 1.5\%$ ($n = 2, 4$ and 3); Hb, 22.3 ± 0.3 , 23.7 ± 0.1 and 24.7 ± 1.3 g dL⁻¹ ($n = 2, 4$ & 3) and Mb, 4.3 ± 0.4 , 4.4 ± 0.6 and 4.6 ± 0.5 g/100 g ($n = 2, 3$ and 3) respectively. Neither Pv, Bv nor total oxygen stores followed the same pattern. Linear regres-

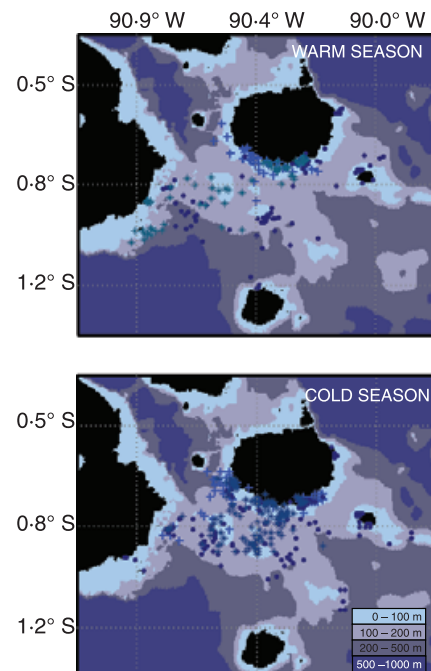


Fig. 3. Satellite locations of female Galapagos sea lions from Caamaño Islet instrumented during a warm and a cold season [March 2005, Villegas-Amtmann *et al.* (2008) and August–September 2006]. $N = 9$ for each season. Different symbols show the three dive type groups.

sion of ungrouped individuals' dive duration and Hb showed a significant relationship ($r^2 = 0.535$, $P = 0.02$; Table 3, Fig. 4).

We found three foraging patterns in the cold season similar to those found in the warm season (Villegas-Amtmann *et al.* 2008). During the cold season individuals from the different groups also exhibited distinct and increasing mean dive durations. Deep divers from the cold season presented the longest dive duration in contrast to bottom divers during the warm season. Mean dive durations for the cold season were 2.87 ± 0.35 ($n = 2$), 4.06 ± 0.22 ($n = 3$) and 5.53 ± 1.47 ($n = 4$) min for the shallow, bottom and deep divers respectively (Table 1). Muscle oxygen stores (Mb) of these groups exhibited trends that increased according to dive durations: Mb, 4.6 , 4.9 ± 0.7 and 5.6 ± 0.9 g/100 g ($n = 1$, 3 and 4) respectively. Pv, Bv and total oxygen stores did not follow the same pattern. Hct and Hb were consistently similar across groups. Linear regression of ungrouped individuals' dive duration and Hb during this season showed a significant relationship ($r^2 = 0.499$, $P = 0.05$; Table 3, Fig. 4).

Across the three groups Hct and Mb values were higher while Hb was consistently lower during the cold season compared to the warm one (Fig. 4). Mean dive depth and duration did not change between seasons (t -test $P = 0.97$ and 0.45 for the warm and cold season respectively). Mean dive duration data were log transformed to acquire normality.

Mean Pv, Bv, Hb and MCHC of all females, including those where diving data were not obtained, were significantly higher during the warm season than the cold one: Pv, 78.4 ± 12.3 mL kg⁻¹ ($n = 8$) and 66.7 ± 10.7 mL kg⁻¹ ($n = 9$) (t -test $P = 0.05$), Bv, 186.0 ± 30.4 mL kg⁻¹ ($n = 8$) and 146.2 ± 36.5 mL kg⁻¹ ($n = 9$) (t -test $P = 0.03$), Hb, 23.4 ± 1.8 g dL⁻¹ ($n = 11$) and 19.6 ± 0.9 g dL⁻¹ ($n = 11$) (t -test $P < 0.001$) and MCHC, 0.4 ± 0.02 ($n = 11$) and 0.3 ± 0.01 ($n = 11$) (t -test $P < 0.001$). In contrast, muscle oxygen stores (Mb) were significantly higher during the cold season than during the warm season: Mb: 5.3 ± 0.4 g/100 g ($n = 11$) and 4.5 ± 0.4 g/100 g ($n = 10$) (t -test $P = 0.01$). Hct values remained the same across both seasons: warm, $57.7 \pm 2.6\%$ ($n = 11$) and cold, $57.7 \pm 2.2\%$ ($n = 11$) (t -test $P = 0.79$). Total body oxygen stores were significantly higher during the warm season (77.4 ± 6.2 , $n = 7$) compared to the cold season (61.5 ± 7.6 , $n = 8$) (t -test $P = 0.01$) (Fig. 5). A mass vs. dive duration linear regression shows a non-significant relationship for both seasons (Fig. 6).

HABITAT UTILIZATION AND FORAGING TRIPS

Satellite tracking duration varied from 11 to 44 days (mean of 18.2 ± 9.7 days) generating 69.1 ± 26.4 (range 38–137) filtered satellite locations for each female ($n = 12$), of which $56.05 \pm 15.2\%$ were at sea ($n = 9$, determined by

Table 3. *Zalophus wollebaeki*. Dive strategies, oxygen stores: haematocrit (Hct), haemoglobin (Hb), plasma volume (Pv), blood volume (Bv), myoglobin (Mb), total oxygen stores and mass. Caamaño Islet, Galapagos, Ecuador. P values show significant differences (t -test) between seasons for the season in which values were greater

| ID | Dive strategy | Hct (%) | [Hb] (g dL ⁻¹) | Pv (mL kg ⁻¹) | Bv (mL kg ⁻¹) | Mb (g/100 g) | Total O ₂ stores (mL kg ⁻¹) | Mass (kg) |
|-----------------------------------|---------------|---------|----------------------------|---------------------------|---------------------------|--------------|--|-----------|
| Warm season March 2005 | | | $P < 0.001$ | $P = 0.05$ | $P = 0.03$ | | $P = 0.01$ | |
| CAAF05 | Shallow | 55 | 22.1 | 80.6 | 183.1 | 4 | 69.8 | 55 |
| CAAF06 | Shallow | 56 | 22.5 | X | X | 4.5 | X | 61.8 |
| CAAF01 | Deep | 53 | 22.6 | 88.4 | 188.1 | 5.0 | 76.4 | 78 |
| CAAF02 | Deep | 62 | 24.9 | 81.0 | 213.2 | 4.3 | 86.7 | 78 |
| CAAF03 | Deep | 59 | 23.6 | 96.9 | 236.3 | X | X | 95.6 |
| CAAF09 | Deep | 58 | 23.7 | X | X | 3.9 | X | 71.6 |
| CAAF08 | Bottom | 57 | 23.3 | 84.8 | 197.2 | 4.8 | 79.1 | 72.6 |
| CAAF10 | Bottom | 60 | 25.8 | X | X | 4.0 | X | 78.2 |
| CAAF11 | Bottom | 59 | 24.9 | 68.9 | 167.9 | 5.0 | 75.0 | 75.4 |
| CAAF04 | X | 56 | 19.3 | 61.1 | 138.8 | 5.3 | 58.3 | 72.4 |
| CAAF07 | X | 60 | 24.8 | 65.3 | 163.3 | 4.1 | 70.6 | 98.4 |
| Cold season August–September 2006 | | | | | | $P = 0.01$ | | |
| CA AFC01 | Shallow | 57 | 19.5 | X | X | X | X | 80.5 |
| CA AFC04 | Shallow | 60 | 19.4 | 55.3 | 138.3 | 4.6 | 55.7 | 75.5 |
| CA AFC03 | Bottom | 58 | 20.1 | 59.0 | 140.5 | 4.8 | 58.1 | 77.5 |
| CA AFC05 | Bottom | 58 | 19.9 | 87.6 | 100.3 | 5.7 | 52.3 | 62 |
| CA AFC12 | Bottom | 59 | 20.3 | X | X | 4.3 | X | 67 |
| CA AFC02 | Deep bottom | X | X | 52.4 | 87.3 | 5.9 | X | 92 |
| CA AFC07 | Deep bottom | 60 | 19.6 | 75.0 | 187.5 | 6.0 | 72.7 | 81.5 |
| CA AFC09 | Deep bottom | 55 | 19.1 | 66.3 | 147.3 | 6.4 | 64.2 | 92 |
| CA AFC10 | Deep bottom | 60 | 21.2 | 69.6 | 173.9 | 4.2 | 66.1 | 78 |
| CA AFC06 | X | 58 | 19.3 | X | X | 4.8 | | 78 |
| CA AFC08 | X | 53 | 17.5 | 67.6 | 143.8 | 5.8 | 58.1 | 62.5 |
| CA AFC11 | X | 57 | 19.4 | 67.6 | 157.2 | 5.9 | 65.3 | 83 |

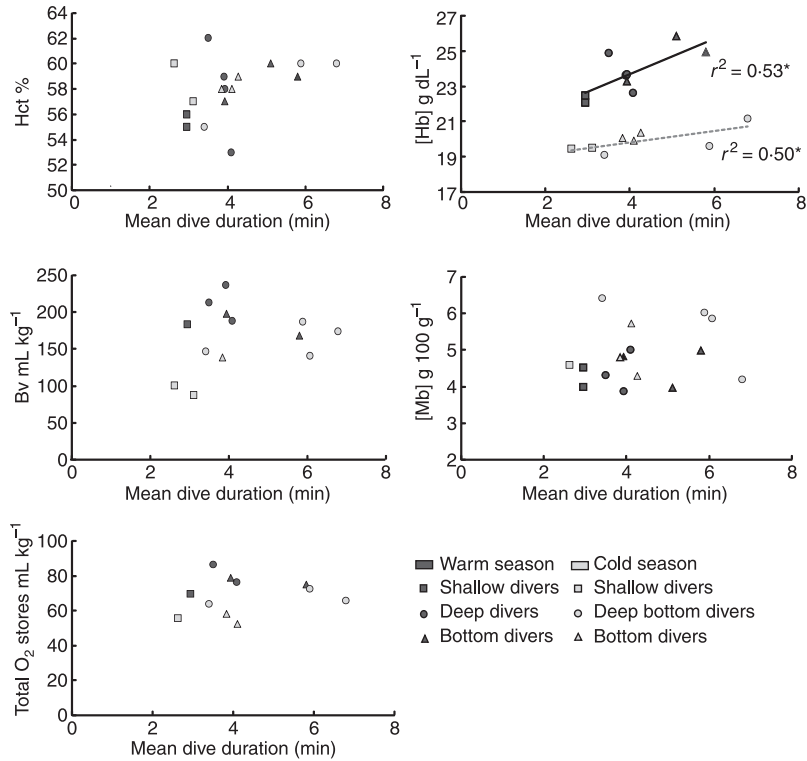


Fig. 4. Linear regressions of mean dive duration and haemoglobin (Hb) (SYSTAT 10.2) of Galapagos sea lion females, Caamaño Islet. Different shapes show different foraging strategies found during a warm season (March 2005) in dark colour and a cold season (August–September 2006) in lighter colour. *Statistical significance.

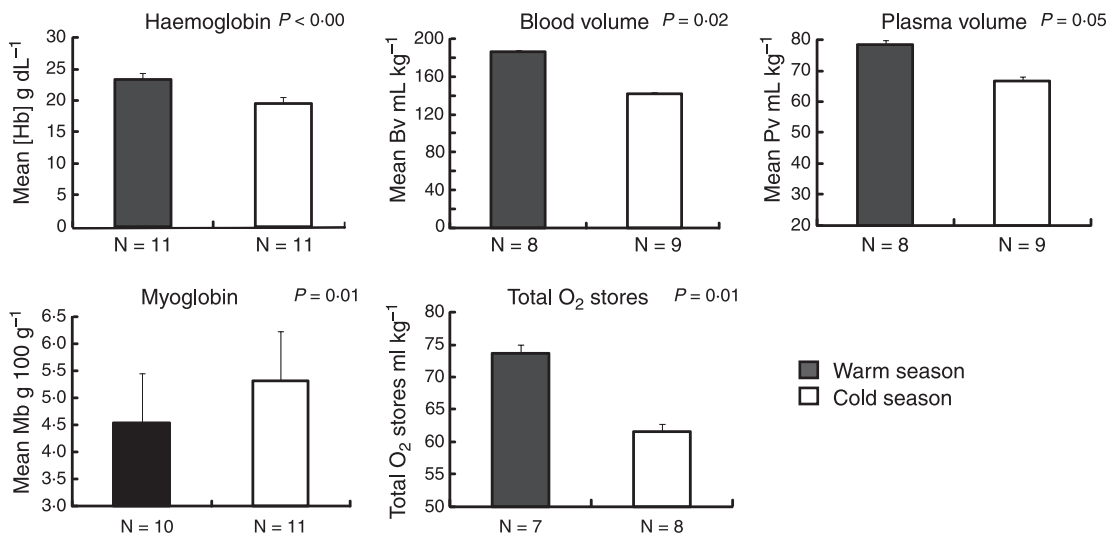


Fig. 5. Galapagos sea lion females mean dive depth, dive duration, haematocrit, haemoglobin, blood volume, plasma volume, myoglobin and total oxygen stores for a warm and cold season (March 2005 and August–September 2006) at Caamaño Islet. *P*-values (*t*-test) are shown at the top right.

haul-out periods in conjunction with TDRs). Females performed 10.56 ± 2.8 unique trips to sea (9–17 trips, $n = 9$ females), spending $59.34 \pm 6.73\%$ of their time at sea. Average maximum distance recorded from the rookery per female was 53.31 ± 24.58 km (range: 8.52–96.74 km, $n = 9$ females).

Foraging trips (time at-sea between haul-out periods) lasted 18.75 ± 6.99 h on average ($n = 9$ females) (range 1.35–49.03 h, $n = 95$ trips). Haul-out times between foraging trips ranged between 1.67 and 39.35 h ($n = 86$ haul-out periods) with a mean of 12.64 ± 3.26 h ($n = 9$ females). Trip durations from the rookery, or the time away from their pups,

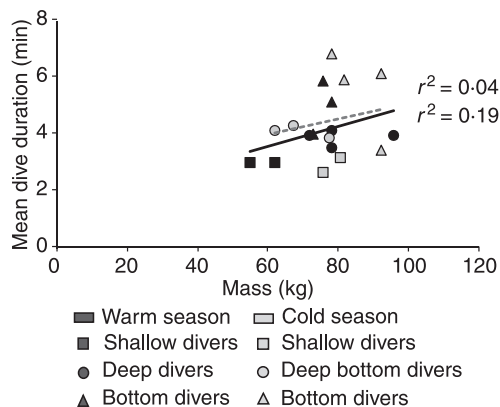


Fig. 6. Linear regression of mass and mean dive duration (SYSTAT 10.2) of Galapagos sea lion females, Caamaño Islet. Different shapes show different foraging strategies found during a warm season (March 2005) in dark colour and a cold season (August–September 2006) in lighter colour. No significant relationship was found.

ranged between 2.17 h and 14.42 days ($n = 22$ trips from rookery), with a mean duration of 6.56 ± 4.7 days (mean range 1.8–14.4 days, $n = 9$ females). Mean haul-out duration at the rookery was 14.54 ± 11.9 h and mean haul out duration at other sites was 10.75 ± 3.4 h.

Females foraged in the same areas during both seasons (Fig. 3). Mean maximum distance travelled (t -test $P = 0.4$), foraging trip duration (t -test $P = 0.2$), haul-out duration (t -test $P = 0.6$), trip duration from the rookery (t -test $P = 0.3$), haul-out duration at the rookery (t -test $P = 0.8$) and at other sites (t -test $P = 0.6$) were all not significantly different between seasons.

Discussion

FORAGING STRATEGIES AND HABITAT UTILIZATION – BOTH SEASONS

Galapagos sea lion females exhibited three foraging strategies during the cold season, similar to those previously found during the warm season: shallow, deep and bottom divers (Villegas-Amtmann *et al.* 2008). Minor differences were observed in the diving behaviour of these three foraging strategies between seasons: The shallow divers during the warm season were the smallest animals and dived primarily during the night. In contrast, shallow divers during the cold season were not the smallest animals and dived during the day and night. The deep divers group dived deeper than during the warm season (warm: 108.2 ± 6.2 and cold: 126.6 ± 47.1 m) in which animals were likely diving mesopelagically (Villegas-Amtmann *et al.* 2008). During the cold season they were diving mostly benthically as they were still foraging in the same areas as during the warm season but were diving deeper, longer, exhibiting longer bottom time duration and a higher percentage of IDZ dives. For this reason they are referred to as 'deep bottom divers' during the cold season.

The persistence of three foraging strategies throughout different seasons with similar diving locations (Figs 2 and 3)

could be an indication of limited food resources in this area of the Galapagos Islands (Martin *et al.* 1994; Sakamoto *et al.* 1998). Our results support the hypothesis of Villegas-Amtmann *et al.* (2008) of the existence of three foraging strategies in GSL during the warm season being indicative of intraspecific competition due to limited resources, supporting the observations of Estes *et al.* (2003) on sea otters (*Enhydra lutris*).

Because females had younger pups during the warm season it was expected that haul-out time and trip durations from the rookery would be shorter during the warm season compared to the cold season. Trip durations from the rookery were shorter during the warm than the cold season (4.18 ± 2 days vs. 6.56 ± 4.7 days respectively). However, these differences were not significant, which could mean that food resources are not abundant enough during the warm season to be able to sustain shorter trip durations from the rookery when pups are younger, as observed in other species (Merrick & Loughlin 1997; Mattlin, Gales & Costa 1998; Beauplet *et al.* 2004); this constraint could be contributing to the vulnerability observed for GSL.

PHYSIOLOGICAL PLASTICITY

The plasticity in oxygen storage capacity of the GSL is demonstrated by the intra and inter-seasonal variability. Intra-seasonally, diving behaviour corresponds to dive physiology as higher oxygen storage capacity (Hb) was found in animals with longer dive durations. Oxygen stores and diving capability of marine vertebrates such as Bottlenose dolphins (*Tursiops truncatus*), Weddell (*Leptonychotes weddellii*), Gray (*Halichoerus grypus*) and Harbour (*Phoca vitulina*) seals, Galapagos fur seals (*Arctocephalus galapagoensis*), California (*Zalophus californianus*), Australian (*Neophoca cinerea*) and Galapagos sea lions, King (*Aptenodytes patagonicus*) and Emperor (*Aptenodytes forsteri*) penguins are known to increase during development and to vary between age and sex classes (Burns & Castellini 1996; Horning & Trillmich 1997; Ponganis *et al.* 1999; Noren *et al.* 2001; Burns *et al.* 2005; Noren, Iverson & Boness 2005; Fowler *et al.* 2007; Weise & Costa 2007; Trillmich *et al.* 2008). Enhanced blood oxygen stores by dive-conditioning have been observed in muskrats (*Ondatra zibethicus*), Harbour seals and tufted ducks (*Aythya fuligula*) (Kodama, Elsner & Pace 1977; Stephenson, Turner & Butler 1989; MacArthur, Weseen & Campbell 2003). Furthermore, changes in oxygen tension can initiate changes in the expression of a variety of genes. Hepatoma cells produce a hypoxia-inducible factor HIF-1 nucleoprotein with the same time course as erythropoietin formation (Jelkmann 1992). HIF-1 has been shown to bind to the 3'-flanking enhancing regions of the erythropoietin gene (Wang & Semenza 1993).

Physiological changes between seasons revealed opposing trends. During the warm season Hb and Bv were significantly higher while Mb was significantly lower compared to the cold season. Although Hct was observed to vary between foraging strategies during the warm season, there was no significant

change in the mean values between seasons. Changes in blood oxygen storage capacity between seasons are not attributed to dive behaviour because animals exhibited similar dive behaviour between seasons.

Pregnancy is associated with major physiological adjustments in mammals and could be contributing to the observed seasonal physiological changes in the GSL. In our study nine of 12 females during the cold season were in a late pregnancy status (~7 months pregnant) (Villegas-Amtmann, Atkinson & Costa 2009). These females face a higher oxygen demand to supply the foetus especially under hypoxic conditions when diving. Maternal perfusion of the placenta continues during diving in Weddell seals (Elsner, Hammond & Parker 1970; Liggings *et al.* 1980). This higher oxygen demand could be reflected in an overall increase in the females' oxygen store increase during the cold season. In contrast, we observed a decrease in Bv and Hb concentration during this season.

The apparent decrease observed in Bv during the cold season when most of the females were pregnant, compared to the warm season, is contrary to that found in humans and rabbits as plasma volume increases up to 50% during a healthy pregnancy (Prince 1982; Longo 1983; Hytten 1985; Letsky 1995). This decrease in Bv could be an artefact because the Evan's blue dye does not cross the placenta (Whipple *et al.* 1955). Therefore, our Bv measurements only represent the mother and do not include the Bv of the foetus. As our Bv measurements were determined relative to the total mass of the mother, which includes the foetus, the total Bv for that mass (female and foetus) would be underestimated. In an attempt to correct for this we subtracted 5 kg (estimated foetal mass; J.B.W. Wolf, pers. comm.) from the females' mass and Bv values remained lower during the cold season, but the differences were not statistically significant. Furthermore, females gain body weight during pregnancy in addition to foetal mass which would also affect mass-specific blood volume calculations. Lastly, it is important to note that although there were a greater number of pregnant females during the cold season, there were also pregnant females during the warm season (Villegas-Amtmann, Atkinson & Costa 2009).

As previously mentioned there were no changes in the sea lions' diving behaviour (dive duration) between seasons but there was a decrease in the blood oxygen stores during the cold season, which was due to a decrease in Hb with no change in Hct. However, reductions in blood oxygen stores were compensated for by an increase in muscle oxygen stores (Mb). This shift from blood to muscle oxygen stores may be related to the increased oxygen demands of a growing foetus. Furthermore, it may also be indicative of iron deficiency anaemia. Such anaemia may be associated with iron limitation in their diet, and or an increased demand for iron during pregnancy (Halvorsen & Halvorsen 1973; Hallberg 1988).

Total oxygen stores changed between seasons as well as the relative contribution of the different oxygen stores. During the warm season we found 8.2%, 66.9% and 24.9% of total oxygen stored in lung, blood and muscle, while during the cold season it was 9.8%, 54.6% and

33.6% respectively. Regardless, the relative distribution of oxygen in the tissues of GSL is consistent with a deep-prolonged diver like a Weddell seal (5%, 66%, 29%) or a sperm whale (*Physeter macrocephalus*) (10%, 58%, 34%) in contrast to that reported for a 'typical' otariid the California sea lion (21%, 45%, 34%) (Kooyman & Ponganis 1998). Furthermore, GSL Hb and Mb values found are the highest reported for an otariid and amongst the highest reported for any diving vertebrate (Kooyman 1989; Costa, Gales & Crocker 1998; Kooyman & Ponganis 1998; Noren & Williams 2000; Ponganis & Kooyman 2000; Fowler *et al.* 2007; Weise & Costa 2007).

In this study our objectives were to determine if the GSL diving behaviour and physiology change as a response to seasonal changes and environmental conditions. Sea lions did not exhibit a change in their dive behaviour between seasons but did within seasons. We found plasticity in the diving physiology of the GSL in conjunction with its dive behaviour within seasons. We found seasonal changes in the sea lions' physiology mainly attributed to pregnancy. To our knowledge this is the first time that a physiological response (oxygen stores) to behavioural differences (dive behaviour) has been observed in the same sex and age class marine mammal or diving vertebrate.

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