Revista de Biología Marina y Oceanografía
ISSN: 0717-3326
revbiolmar@gmail.com
Universidad de Valparaíso
Chile

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Revista de Biología Marina y Oceanografía, vol. 50, núm. 1, abril, 2015, pp. 81-93
Universidad de Valparaíso
Viña del Mar, Chile

Revista de Biología Marina y Oceanografía
Vol. 50, Nº1: 81-93, abril 2015

ARTICLE

Biochemical and hematological profile of *Otaria flavescens* in the reproductive colony of Cobquecura, central-south Chile

Perfil bioquímico y hematológico de *Otaria flavescens* en la colonia reproductiva de Cobquecura en Chile centro-sur

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Resumen.- A pesar de la importancia ecológica del lobo marino común *Otaria flavescens*, existe escasa información referente a su química sanguínea y hematología, los cuales pueden ser utilizados para evaluar el estado de salud de las poblaciones. El presente trabajo, reporta por primera vez la determinación de 14 parámetros químicos sanguíneos de *O. flavescens* y su fórmula leucocitaria obtenida desde 15 individuos pertenecientes a una colonia reproductiva localizada en Cobquecura, zona centro-sur de Chile, durante 2 muestreos realizados, 2009 y 2011. Los resultados indican que existe una alta regularidad bioquímica para los parámetros estudiados. No obstante, se presentan diferencias significativas en la concentración plasmática de calcio entre ambos sexos y entre las concentraciones promedio de proteínas totales, globulinas, colesterol y actividad de colinesterasa de los 2 muestreos estudiados. Comparado con otros perfiles bioquímicos reportados para mamíferos marinos, en esta especie se determinó una alta concentración de globulinas y capacidad inhibitoria de antiproteasa (cercana al 100%), una moderada a baja actividad de colinesterasa y una concentración de glucosa en el rango de lo ya reportado. Finalmente, la fórmula leucocitaria muestra una eosinófilia moderada a alta para el primer muestreo analizado (8,3-17,6%), lo que sugiere la posible presencia de alguna parasitosis en la población estudiada. La presente información puede ser útil para la determinación del estado de salud de poblaciones de *O. flavescens*, y por lo tanto, podría contribuir a la conservación de la especie en el Pacífico Sur Oriental.

Palabras clave: *Otaria flavescens*, lobo marino común, química sanguínea, hematología, fórmula leucocitaria

Abstract.- Despite the ecological importance of the common sea lion *Otaria flavescens,* there is currently no information available on the blood chemistry and hematology of this species. Here, we report for the first time, 14 blood chemistry variables of *O. flavescens* and differential blood cell counts obtained from 15 resident individuals from a reproductive colony located in Cobquecura on the coast of central-south Chile, during 2 sampling periods, in 2009 and 2011. There was a high degree of biochemical regularity in studied parameters, although the plasmatic concentration of calcium differed significantly between genders; average concentrations of total proteins, globulins, cholesterol, and cholinesterase activity varied significantly between sampling periods. In comparison with the biochemical profiles reported for other marine mammals, *O. flavescens* had greater concentrations of globulins; an inhibitory capacity of antiproteases (close to 100%); and moderate to low cholinesterase activity and similar concentrations of glucose. Finally, differential blood cells counts indicated moderate to high eosinophilia (8.3-17.6%) in individuals sampled during the first sampling period, which suggests the possible presence of parasitism within this population. These results could be useful for assessing the state of health of *O. flavescens* populations and, therefore, contribute to species conservation in the Southeast Pacific.

Key words: *Otaria flavescens*, South American sea lion, blood chemistry, hematology, differential leukocyte counts

INTRODUCTION

The South American sea lion, *Otaria flavescens* (Shaw, 1800), is one of the 5 species belonging to the subfamily Otariinae (family Otariidae). It is the only genera of the *Otaria* (Riedman 1990, Cappozzo & Perrin 2009). Its spatial distribution includes the coasts of South America, with relatively abundant populations from northern Peru (Scheffer 1958, Riedman 1990) to the Diego Ramírez Islands in the Austral Pacific off Chile (Osgood 1943, Schlatter & Riveros 1997). Along the Atlantic coast, this species is distributed from the south of Torres in Brazil (Rosas *et al.* 1994) to Tierra del Fuego in Argentina (King 1983, Dans *et al.* 2012), as well as the Falkland/Malvinas and Isla de los Estados (Osgood 1943, Bastida & Rodríguez 2003). Typically piscivorous (Vaz-Ferreira 1982, Crespo *et al.* 1997), this species has a varied diet including crabs, cephalopods (mollusks), squid and gastropods (Aguayo & Maturana 1973, George-Nascimento *et al.* 1985, Sielfeld *et al*. 1997, Aguayo-Lobo *et al*. 1998, Hückstädt & Antezana 2006, Hückstädt *et al.* 2007, De la Torriente *et al.* 2010, Muñoz *et al.* 2013). However, the main dietary items of these animals are largely determined by the prey species available in their habitat, making them opportunistic predators (Sielfeld *et al.* 1997, Koen-Alonso *et al.* 2000).

Chile hosts the greatest abundance of South American sea lions in the world, with a population of approximately 150,000 individuals (Sielfeld *et al.* 1997, Aguayo-Lobo *et al*. 1998, Oporto *et al.* 1999, Venegas *et al.* 2001, Sepúlveda *et al*. 2007, Bartheld *et al.* 2008, Oliva *et al.* 2012). While overall population estimates have increased steadily over the past decade, certain populations appear to be affected by marine pollution and a decrease in prey abundance due to the overexploitation of fisheries (Venegas *et al.* 2001, Sepúlveda *et al.* 2011).

Previous research efforts on this species have focused on determining abundance and population distribution (*e.g.*, Sielfeld *et al.* 1997, Aguayo-Lobo *et al.* 1998, Bartheld *et al.* 2008, Sepúlveda *et al.* 2011). However, there are scarce data on blood biochemistry and hematology (Ikehara *et al.* 1996). The assessment of blood chemistry and hematology profiles allows the determination of analytes that indicate the state of health and development of these animals (Roletto 1993), taking into account various physiological processes (Panfoli *et al.* 1999, Lehninger *et al.* 2001, Yuan *et al.* 2001). These profiles vary with the sex, developmental stage, and physical condition of an individual (Urich 1994, Sterner & Elser 2002).

In this study, we report for the first time 14 blood chemistry parameters and hemograms obtained from 15 *O. flavescens* individuals belonging to the breeding colony at Cobquecura. This is the most important breeding colony of the central zone of Chile, with approximately 2,500 individuals (Sepúlveda *et al.* 2011).

MATERIALS AND METHODS

Fifteen sea lions between 115 to 165 cm length, including 10 juveniles, four sub-adult males, and one adult female, were sampled during July 2009 (n= 6) and April 2011 (n= 9) from the coastal area of the Nature Sanctuary of Islote Lobería de Cobquecura (36°0.7’53’’S-72°48’29’’W), which has been state protected since 1992, within the Biobío Region of central southern Chile (Fig. 1).

During the sardine fishery catch, sea lions take advantage of the aggregation of small pelagic driven by purse seine nets to feed freely. This provides an ideal opportunity to catch individuals for sampling. Individuals were captured with a lasso in the water and immediately transferred to a mesh net on board a small purse seine vessel belonging to the sardine fishing fleet, just outside the waters of the Nature Sanctuary. Animals immobilized in the net were then transferred to a small artisan fishing vessel (8 m length) and taken to land. Capture times were never more than 20 min per animal, from first contact with the lasso to securing the animal in the small boat. The time elapsed between securing the animal in the boat and reaching land was approximately 15 min. During the procedure, animals were monitored by a veterinarian, and only apparently healthy animals were selected for sampling; we considered healthy animals to be those which were found without wounds and with sufficient strength to resist capture. On land, animals were anaesthetized using isoflurane gas (0.5 a 2.5%) with oxygen, administered using a conical mask and a portable vaporizer (Gales & Mattlin 1998). Once the animals were anaesthetized, they were weighed using a stretcher and tripod attached to a digital scale (± 0.1 kg) and total length was determined with a flexible measuring tape from the tip of the nose to the tip of the tail (standard length, cm). Given logistical difficulties during the second sampling campaign, it was not possible to obtain the body weights of all individuals.

Blood samples were taken via puncture of the caudal gluteal vein with a heparinized syringe. Afterwards, animals were allowed to recover from anesthesia while being monitored, and were then released a few meters

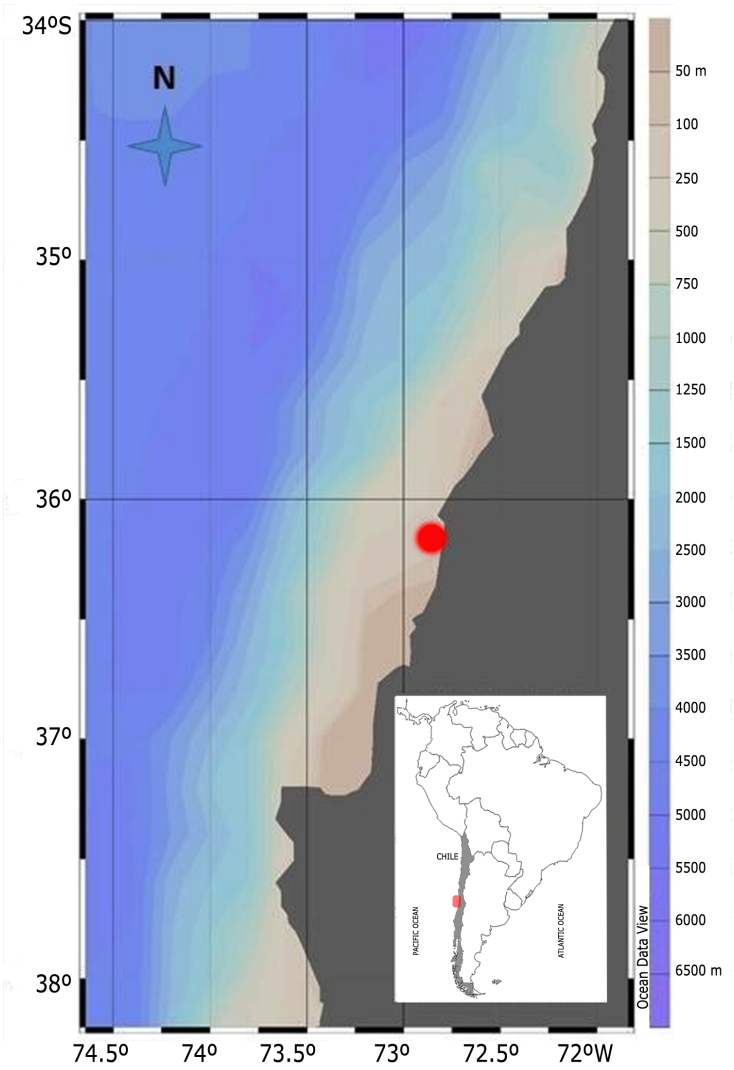

Figure 1. Study area, with the Nature Sanctuary of Islote Lobería de Cobquecura marked by a circle / Zona muestreo Santuario de la Naturaleza Islote Lobería de Cobquecura, señalada con un círculo

from the sea. All animals survived this sampling procedure. Blood samples were immediately centrifuged at 1000 x *g* x 10 min (4ºC) to separate the plasma and the formal elements in the blood, and plasma samples were stored at -20ºC until analysis.

Blood chemistry analysis

Biochemical profiles included albumin, total protein, phosphorus, calcium, lactate, glucose, cholesterol, urea, cholinesterase, lactate dehydrogenase, alanine aminotransferase (ALT), aspartate transaminase (AST), lysozyme and inhibitory activity of antiprotease were analyzed using a combination of methodologies.

The analysis of albumin, total proteins, phosphorus, calcium, glucose, cholesterol, urea, ALT and AST were determined according to the protocol of clinical analysis based on kits by the company Human Diagnostics (Wiesbaden, Germany).The activity of cholinesterase (ChE) and lactate were determined according to the clinical analysis kits protocol established by the company Sentinel Diagnostics (Milan, Italy).

The activity of the catabolic lactate dehydrogenase enzyme (LDH) was measured using a modified reaction mix from the Schiedek (1997) method, which contained a buffer of K_2HPO_4, NADH and pyruvate for LDH. Reactions were initiated with the addition of an aliquot of

the supernatant, and the decay in NADH absorption was measured to 340 nm. Enzymatic activity was corrected by the unspecific oxidation of NADH.

Lysozyme activity was determined using a method based on the lysis of *Micrococcus lysodeikticus*, according to Ellis (1990a). A unit (U) of lysozyme was defined as the quantity of enzyme necessary to cause a decrease in the absorbance by 0.001 units per min. Lysozyme activity was standardized to U mL^{-1} plasma. A standard egg lysozyme calibration curve was constructed, which allowed transforming the data to concentrations of lysozyme per unit volume of plasma (μg mL^{-1}).

The antiprotease activity of plasma (capacity of trypsin inhibition by alfa-2-macroglobulin) was quantified using a modified essay by Ellis (1990b) and Zuo & Woo (1997), which is based on the ability of the antiprotease of the study organism to inhibit the activity of trypsin. The inhibitory capacity of trypsin was expressed as the percentage difference between the absorbance of the control (without plasma, without inhibition) minus the absorbance of the sample (which contains antiprotease activity), divided by the absorbance of the control.

Hematology

In parallel to blood chemistry analyses, blood smears were made to determine the differential blood count. Smears were dyed with May-Grunwald-Giemsa. Differential blood cells (*i.e.*, monocytes, eosinophils, lymphocytes, polymorphonuclears, leukocyte bacilliforms and basophils) were counted using an optical microscope with 40X magnification.

Statistical analyses

Considering the small sample size, we decided to use the non-parametric Mann-Whitney test (IBM SPSS Statistics 20) to determine whether the blood variables differ significantly between the first and second sampling periods, as well as between genders ($P < 0.05$).

Results

Blood chemistry analysis

Standard length of sea lions (n= 11 males and 4 females) ranged between 115 cm and 165 cm, and mass ranged between 49 kg and 116.2 kg. No differences in blood chemistry parameters were detected between genders, with the exception of calcium, which was lower in females than in males (7.8 ± 1.1 and 10.4 ± 2.0 mg dL^{-1}, respectively; $P < 0.05$) considering both samplings dates.

The data presented in Table 1 shows mean values ± SD, and also includes the data range for each of the blood chemistry parameters analyzed during each sampling period (2009 and 2011). During the first sampling period, significantly higher concentrations were found for total proteins, globulins, and cholesterol; thus, the activity of

Table 1. Mean values, standard deviation and data ranges of blood chemistry of the South American sea lion, *Otaria flavescens*, obtained in the coastal area of the Nature Sanctuary of Islote Lobería de Cobquecura (36°0.7´53´´S-72°48´29´´W). The samplings were made in July 2009 (n= 6) and April 2011 (n= 9) / Valores promedio, desviación estándar y rango de datos para química sanguínea de lobo marino Sur Americano *Otaria flavescens* recolectados en la costa del área del Santuario de la Naturaleza del Islote Lobería de Cobquecura (36°0,7´53´´S-72°48´29´´W). Los muestreos fueron realizados en julio 2009 (n= 6) y abril 2011 (n= 9)

Parameters	Sampling 1	Data Range Sampling 1	Sampling 2	Data Range Sampling 2
Albumin (g dL^{-1})	4.3 ± 2.4	1.8 - 7.9	4.1 ± 1.1	1.6 - 5.4
Total proteins (g dL^{-1})	25.5 ± 8.3*	14.5 - 34.3	8.1 ± 2.1	4.1 - 10.8
Globulins (g dL^{-1})	21.2 ± 9.3*	9.4 - 31.4	4.1 ± 1.3	1.8 - 5.6
Phosphorus (mg dL^{-1})	10.7 ± 7.2	4.7 - 23.9	5.2 ± 1.4	2.9 - 7.3
Calcium (mg dL^{-1})	12.5 ± 3.3	8.7 - 17.8	8.6 ± 1.1	6.7 - 10.2
Lactate (mg dL^{-1})	71.7 ± 15.7	54.6 - 95.5	36.7 ± 15.8	18.4 - 70.7
Glucose (mg dL^{-1})	145.7 ± 23.9	117.0 - 173.6	119.4 ± 24.4	84.9 - 157.3
Cholesterol (mg dL^{-1})	967.9 ± 393.9*	577.3 - 1552.5	223.1 ± 70.3	132.3 - 262.8
Cholinesterase (UI L^{-1})	441.3 ± 182.3*	131.6 - 592.2	815.6 ± 125.6	592.2 - 954.1
LDH (IU L^{-1})	280 ± 0.1	155.0 - 412.6	n.d.	n.d.
Lysozyme (IU mL^{-1})	119.1 ± 65.4	50.0 - 180.0	n.d.	n.d.
Inhibitory Antiprotease Activity (%)	96.6 ± 2.3	94.4 - 99.0	n.d.	n.d.
Urea (mg dL^{-1})	90.4 ± 9.9	86.3 - 100.2	94.1 ± 24.5	66.8 - 131.4
AST (IU L^{-1})	19.1 ± 6.1	13.1 - 24.4	21.3 ± 12.8	7.9 - 49.1
ALT (IU L^{-1})	22.4 ± 7.6	14.7 - 32.3	10.2 ± 6.9	0.9 - 20.1
AST/ALT	1.0 ± 0.6	0.5 - 1.7	7.8 ± 12.7	0.5 - 32.0

n.d. = no data; * $P < 0.05$

the cholinesterase was found to be less than during the second sampling period (Table 1). Also, during the first sampling period, a high inhibitory capacity of antiprotease was recorded, almost 100%, although this parameter, as well as LDH and lysozyme, was not determined during the second sampling period.

An interesting finding was the ratio between cholesterol/cholinesterase relative to the body length of individuals, where a constant value of this independent length ratio was observed, with the exception of one individual, which lay absolutely outside the range due to its low cholinesterase activity (Fig. 2).

Hematology

The leucocyte range obtained for this species during both sampling periods is presented in Table 2. The most abundant circulating cells in the blood of this species were polymorphonuclears (PMN) and lymphocytes. Significant differences were observed ($P < 0.05$) between the first and second sampling periods for eosinophils, polymorphonuclears, monocytes, and basophils.

All individuals analyzed during the first sampling period had moderate to high eosinophilia (range= 8.3 to 17.6%). In contrast, during the second sampling period, individuals presented a low to moderate percentage of eosinophils, between 2.5 and 9.9%.

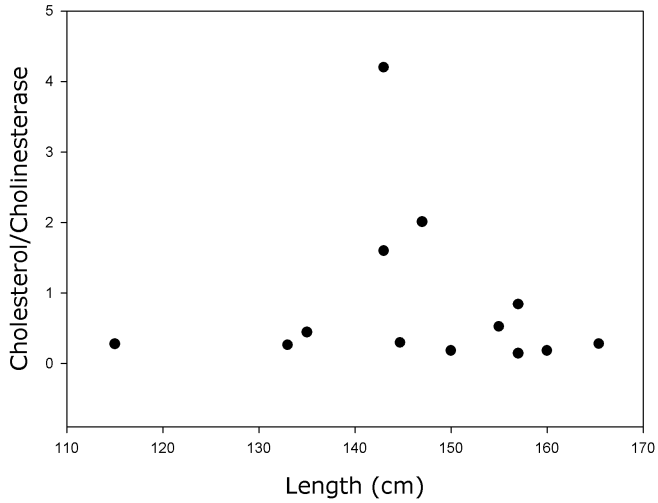

Figure 2. Relationship between body length of individuals and the cholesterol/cholinesterase activity ratio / Relación entre longitud de los individuos y la razón colesterol/actividad de colinesterasa

Table 2. Mean values, standard deviation and data range of differential blood cell counts of the South American sea lion *Otaria flavescens* collected in the coastal area of the Nature Sanctuary of Islote Lobería de Cobquecura (36°0.7´53´´S-72°48´29´´W). Sampling was carried out in July 2009 (n= 6) and April 2011 (n= 9) / Valores promedio, desviación estándar y rango de datos para la fórmula leucocitaria de lobo marino Sur Americano *Otaria flavescens* recolectados en la costa del área del Santuario de la Naturaleza del Islote Lobería de Cobquecura (36°0.7´53´´S-72°48´29´´W). Los muestreos fueron realizados en julio 2009 (n= 6) y abril 2011 (n= 9)

Parameter	Sampling 1	Data Range Sampling 1	Sampling 2	Data Range Sampling 2
Polymorphonuclear (%)	45.8 ± 2.1	41.4 - 50.8	53.0 ± 4.2*	46.6 - 59.6
Lymphocyte (%)	32.9 ± 2.4	24.6 - 41.3	36.0 ± 5.0	30.1 - 42.7
Eosinophil (%)	11.9 ± 3.3*	8.3 - 17.6	6.5 ± 2.3	2.5 - 9.9
Monocyte (%)	8.7 ± 1.0*	6.4 - 12.4	3.2 ± 1.0	1.1 - 4.7
Leukocytebacilliforms (%)	0.6 ± 0.3	0.3 - 0.7	0.2 ± 0.5	0.0 - 1.5
Basophil (%)	0.2 ± 0.3	0.0 - 0.4	0.8 ± 0.4*	0.3 - 1.4

*$P < 0.05$

Discussion

To the best of our knowledge there has been no published information regarding the blood chemistry and differential blood cell counts for *Otaria flavescens* prior to this study. For the most part, our results revealed biochemical consistencies between genders and sampling periods (Table 1). Although calcium differed between females and males in this study, the values were still within ranges reported for other marine mammals (Table 3), including otariids. Average values obtained for albumin and phosphorus were also similar to those reported in the literature (Table 3). These values were also consistent with those described by Roletto (1993) for rehabilitated, clinically healthy California sea lions *(Zalophus californianus*), northern elephant seals (*Mirounga angustirostris*), and harbor seals (*Phoca vitulina richardii*), as well as with the ranges described in Bossart *et al.* (2001) for these analytes.

In wild marine mammals, the concentration of glucose in the blood can vary due to age, diet, physical activity, metabolism, environmental conditions, and seasonal variation (Halloran & Pearson 1972, LeResche *et al.* 1974, Seal *et al.* 1975, Matula *et al.* 1980, Colares *et al.* 2000). In addition, a glucose increase in plasma can be the product of stress following the manipulation of animals during sampling (Seal *et al.* 1975, LeResche *et al.* 1974, Hyvarinen *et al.* 1975, Koopman *et al.* 1995). In the present study, glucose concentration was on average higher during both study periods (Tables 1 and 3), in comparison with the California sea lion, which is the otariid species most closely related to *O. flavescens* with available data (Table 3). The values of glucose obtained for *O. flavescens* were similar to those observed in seals and elephant seals (Davis *et al.* 1991, Trumble & Castellini 2002, Roletto 1993, Table 3), as well as to the Steller sea lion (*Eumetopias jubatus*; Lander *et al.* 2014).

Davis (1983) and Davis *et al.* (1991) found lactate values of captive harbor seals that were considerably lower than those reported in this study; however, the lactate values reported by these authors (Table 3) were based on measurements of individuals in a resting state. The elevated values of lactate found for *Otaria* may represent the period of post-immersion recovery of studied individuals, as found for other vertebrates (Hochachka & Somero 1973). Accumulated plasma lactate concentrations in studied individuals (Table 1), when transformed to molar concentrations, are within the range of 4.1 to 7.9 mM. The values were also one order of magnitude greater than reported plasma lactate for the post-diving recovery period (0.55 mM) determined for Weddell seals (Butler & Jones 1997). However, studies conducted by Werner & Campagna (1995), determined that the maximum dive time for *O. flavescens* corresponds to 7.7 min, but these authors did not report the plasma lactate concentration after these dives. It is interesting to note that Weddell seals have longer dive durations than *O. flavescens*, and accumulate less plasma lactate determined in this study. This might imply that sea lions reach higher lactate concentrations more quickly compared to Weddell seals. It was therefore not possible to infer the relationship between the plasma concentrations of this analyte determined in the present study with the dive time observed by Werner & Campagna (1995).

The high value of total proteins from the first sampling period compared with the second sampling period was noteworthy (Table 1). Values from the second period were comparable with values in the literature (Table 3), suggesting an altered physiological state for individuals from the first sampling period, given that total proteins were at least twice as high as those reported for other marine mammals (Table 3). However, the albumin values obtained during both sampling periods were within the same range as those in the literature (Table 3). Given this observation, it appears that globulin proteins contributed to the increase in protein fraction in these animals during the first sampling period. This increase can be explained by the high concentration of lipoproteins necessary to stabilize high values of cholesterol registered from the first sampling period (Table 1), close to the reported lipid range for phocids (1200 to 3000 mg dL^{-1}; Dangerfield *et al.* 1976). However, cholesterol values of *O. flavescens* were greater than those reported for other pinnipeds (Table 3). Values from the second sampling period were lower and within the range of values reported in the literature (Table 3) and displayed significant differences ($P < 0.05$) compared to the first sampling period. A possible explanation is that the principal prey of *O. flavescens* is the anchovy (*Engraulis ringens*), which have a more carnivorous diet during the austral winter (July 2009) compared with the end of the austral summer (April 2011), when it is feeds almost exclusively on phytoplankton (Krautz *et al.* 2012).

The innate immune response is the first line of defense against a broad spectrum of pathogens in the environment and an important physiological mechanism for maintaining homeostasis. Lysozyme is an important molecule of the innate immune response, which mediates protection

Table 3. Average values and standard deviations were obtained for each of the blood chemistry values and differential blood cell counts in *Otaria flavescens*, compared with existing information in the literature on pinnipeds and other marine mammals. Parameter values are listed in ascending order / Valores promedios y desviación estándar obtenidos para cada uno de los parámetros de química sanguínea y fórmula leucocitaria determinado en *Otaria flavescens* comparada con la información existente en la literatura respecto a pinnípedos y otros mamíferos marinos. Los valores de los parámetros están presentados en orden ascendente

Parameter	Species	Value	Reference
Albumin (g dL^{-1})	Gray whale/*Eschrichtius robustus*	1.8 ± n.d.	Dailey *et al.* 2000
	Walrus/*Odobenus rosmarus*	2.2 - 3.1	Bossart et al. 2001
	Harbor seal/*Phoca vitulina*	2.5 - 3.3	Bossart *et al.* 2001
	California Sea Lions/*Zalophus californianus*	2.7 - 3.7	Bossart *et al.* 2001
	Northern elephant seal/*Mirounga angustirostris*	3.2 - 3.9	Bossart *et al.* 2001
	Harbor seal/*Phoca vitulina*	3.39 ± 0.41	Trumble & Castellini. 2002
	Amazonian manatee/*Trichechus inunguis*	3.4 ± 0.1	Colares *et al.* 2000
	Steller sea lion/*Eumetopias jubatus*	3.5 - 4.5	Bossart *et al.* 2001
	California Sea Lions/*Zalophus californianus*	3.8 ± 0.2	Roletto 1993
	Pacific Harbour Seal/*Phoca vitulina richardii*	3.8 ± 0.2	Roletto 1993
	Northern elephant seal/*Mirounga angustirostris*	3.8 ± 0.4	Roletto 1993
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	4.2 ± 1.6	This study
Total protein (g dL^{-1})	Harbor seal/*Phoca vitulina*	0.66 ± 0.08	Trumble & Castellini 2002
	Gray whale/*Eschrichtius robustus*	5.7 ± n.d.	Dailey *et al.* 2000
	Amazonian manatee/*Trichechus inunguis*	6.4 ± 0.1	Colares *et al.* 2000
	Northern elephant seal/*Mirounga angustirostris*	7.2 ± 1.0	Roletto 1993
	Pacific Harbour Seal/*Phoca vitulina richardii*	7.3 ± 0.8	Roletto 1993
	California Sea Lion/*Zalophus californianus*	8.0 ± 1.0	Roletto 1993
	Juan Fernández fur seal/*Arctocephalus philippi*	9.4 ± 1.7	Sepúlveda *et al.* 1999
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	12.6 ± 8.3	This study
Globulin (g dL^{-1})	Harbor seal/*Phoca vitulina*	0.32 ± 0.05	Trumble & Castellini 2002
	Amazonian manatee/*Trichechus inungu*is	3.0 ± 0.1	Colares *et al.* 2000
	Gray whale/*Eschrichtius robustus*	3.9 ± n.d.	Dailey *et al.* 2000
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	10.9 ± 10.4	This study
Phosphorus (mg dL^{-1})	California Sea Lions/*Zalophus californianus*	1.8 - 7.8	Bossart *et al.* 2001
	Harbor seal/*Phoca vitulina*	1.9 - 7.4	Bossart *et al.* 2001
	Gray whale/*Eschrichtius robustus*	4.1 ± n.d.	Dailey *et al.* 2000
	Walrus/*Odobenus rosmarus*	4.4 - 6.4	Bossart *et al.* 2001
	Steller sea lion/*Eumetopias jubatus*	5.3 - 9.1	Bossart *et al.* 2001
	Northern elephant seal/*Mirounga angustirostris*	6.6 - 9.9	Bossart *et al.* 2001
	Harbor seal/*Phoca vitulina*	6.71 ± 1.56	Trumble & Castellini 2002
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	7.4 ± 5.2	This study
Calcium (mg dL^{-1})	California Sea Lion/*Zalophus californianus*	9.4 ± 0.5	Roletto 1993
	Pacific Harbour Seal/*Phoca vitulina richardii*	9.7 ± 0.5	Roletto 1993
	Harbor seal/*Phoca vitulina*	9.9 ± 1.4	Trumble & Castellini 2002
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	10.1 ± 2.9	This study
	Northern elephant seal/*Mirounga angustirostris*	10.4 ± 0.6	Roletto 1993
	Gray whale/*Eschrichtius robustus*	13.7 ± n.d.	Dailey *et al.* 2000
Lactate (mg dL^{-1})	Harbor seal/*Phoca vitulina*	3.96 ± 1.6	Davis 1983
	Harbor seal /*Phoca vitulina*	7.2 ± n.d.	Davis *et al.* 1991
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	50.7 ± 23.3	This study

n.d.= no data

Table 3 continued / Continuación Tabla 3

Parameter	Species	Value	Reference
Glucose (mg dL^{-1})	Gray whale/*Eschrichtius robustus*	14 ± n. d.	Dailey *et al.* 2000
	Amazonian manatee/*Trichechus inunguis*	34.4 ± 2.1	Colares *et al.* 2000
	Short-beaked common dolphins/*Delphinus delphis*	94 ± 22.4	Ortiz & Worthy 2000
	Harbor seal/*Phoca vitulina*	102.6 ± 10.8	Davis 1983
	Weddellseal/*Leptonychotes weddellii*	108.1 ± 10.9	Sakamoto *et al.* 2009
	California Sea Lion/*Zalophus californianus*	113 ± 30	Roletto 1993
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	130.2 ± 26.8	This study
	Pacific Harbour Seal/*Phoca vitulina richardii*	136 ± 25	Roletto 1993
	Northern elephant seal/*Mirounga angustirostris*	144 ± 27	Roletto 1993
	Harbor seal/*Phoca vitulina*	154.8 ± n.d.	Davis *et al.* 1991
	Harbor seal/*Phoca vitulina*	165.9 ± 25.8	Trumble & Castellini 2002
Total cholesterol (mg dL^{-1})	Harbor seal/*Phoca vitulina*	337.2 ± 85.3	Trumble & Castellini 2002
	Gray whale/*Eschrichtius robustus*	339 ± n.d.	Dailey *et al.* 2000
	Weddellseal/*Leptonychotes weddellii*	368 ± 97	Sakamoto *et al.* 2009
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	521.0 ± 448.2	This study
LDH (IU L^{-1})	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	280 ± 0.1	This study
	Gray whale/*Eschrichtius robustus*	409 ± n.d.	Dailey *et al.* 2000
	Northern elephant seal/*Mirounga angustirostris*	556 ± 294	Roletto 1993
	Pacific Harbour Seal/*Phoca vitulina richardii*	811 ± 265	Roletto 1993
	California Sea Lion/*Zalophus californianus*	888 ± 343	Roletto 1993
	Harbor seal/*Phoca vitulina*	3345 ± 1258	Trumble & Castellini 2002
Cholinesterase (IU L^{-1})	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	665.9 ± 238.6	This study
	California Sea Lion/*Zalophus californianus*	815,6 ± 125.6	Donovan *et al.* 1994
Lysozyme (IU mL^{-1})	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	119.1 ± 65.4	This study
Inh.Ant.Cap. (%)	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	96.6 ± 2.3	This study
Urea (mg dL^{-1})	Weddellseal/*Leptonychotes weddellii*	25.9 ± 13.7	Sakamoto *et al.* 2009
	Amazonian manatee/*Trichechus inunguis*	26.6 ± 2.6	Colares *et al.* 2000
	Harbor seal/*Phoca vitulina*	34.9 ± 9.6	Trumble & Castellini 2002
	Gray whale/*Eschrichtius robustus*	58 ± n.d.	Dailey *et al.* 2000
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	90.3 ± 20.7	This study
AST (IU L^{-1})	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	20.6 ± 10.9	This study
	Gray whale/*Eschrichtius robustus*	27.9 ± n.d.	Dailey *et al.* 2000
	Harbor seal/*Phoca vitulina*	75.2 ± 29.9	Trumble & Castellini 2002
ALT (IU L^{-1})	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	14.0 ± 9.0	This study
	Gray whale/*Eschrichtius robustus*	20 ± n.d.	Dailey *et al.* 2000
	Harbor seal/*Phoca vitulina*	24.6 ± 11.7	Trumble & Castellini 2002
AST/ALT	South American sea lion/*Otaria flavescens* (Julio 2009-Abril 2011)	5.7 ± 10.9	This study
	Gray whale/*Eschrichtius robustus*	13.95	Dailey *et al.* 2000
PMN (%)	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	51.1 ± 5.3	This study
	Harbor seal/*Phoca vitulina*	60.2 ± 10.4	Trumble & Castellini 2002
Lymphocytes (%)	Harbor seal/*Phoca vitulina*	28.1 ± 9.7	Trumble & Castellini 2002
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	35.1 ± 5.5	This study
Eosinophils (%)	Harbor seal/*Phoca vitulina*	3.2 ± 2.9	Trumble & Castellini 2002
	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	8.0 ± 3.7	This study
Monocytes (%)	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	4.8 ± 3.0	This study
	Harbor seal/*Phoca vitulina*	8.9 ± 5.3	Trumble & Castellini 2002
Basophils (%)	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	0.6 ± 0.4	This study
	Harbor seal/*Phoca vitulina*	0.9 ± 1.1	Trumble & Castellini 2002
Leukocyte bacilliforms (%)	South American sea lion/*Otaria flavescens* (July 2009-April 2011)	0.3 ± 0.4	This study

n.d.= no data

against microbial invasion. It is present in mucus, secretions, saliva and plasma, and has been utilized as an innate immune response indicator in fish (Callewaert & Michiels 2010, Saurabh & Sahoo 2008). In marine mammals, there are only a few reports of immune response, and skin lysozyme presence has only been reported in *P. vitulina* and *Callorhinus ursinus* (Meyer *et al.* 2003). The importance of these enzymes lies in the presence of microorganisms in the marine environment, which are capable of causing skin lesions, such as dermatitis due to fungi in Californian sea lions (Guillot *et al.* 1998). In this study, we present new data on the plasmatic activity of lysozyme in *O. flavescens* individuals, which did not have any apparent skin lesions when they were captured. Changes in lysozyme activity could be associated with the occurrence of pathogens in mammals (Maraghi *et al.* 2012); in fish (Saurabh & Sahoo 2008), mammals and other animals (Demers & Bayne 1997, Callewaert & Michiels 2010), changes in activity have also been linked to stress-inducing environmental conditions, pollution and seasonal variability.

The diagnostic value of determining the plasma activity of cholinesterase is that it can be inhibited by organophosphorus and carbamate plagicides, as well as by heavy metals and detergents (Guilhermino *et al.* 1998). Therefore, a drop in the activity of this enzyme suggests a degree of adsorption and exposure to some substance that inhibits its activity. Average plasma cholinesterase activity found for captured and rehabilitated Californian sea lions (Donovan & Zinkl 1994; Table 3) was greater than that observed in the plasma during the first sampling period and very close to the activity measured during the second sampling period (Table 1), with a significant difference between both study periods. This may indicate the presence of some inhibitor of this enzyme in the environment during the first sampling period, however, the number of samples (3) in the study by Donovan & Zinkl (1994) and the difference in focal species limits comparison with the present study. Nevertheless, during the first sampling period, the activity of cholinesterase ranged between 131.6 and 636.1 IU L^{-1}. The individual that displayed lowest cholinesterase activity (131.6 IU L^{-1}) also had the highest plasma cholesterol concentration (1552.5 mg dL^{-1}). The ratio of cholesterol/cholinesterase of this individual (male), with a body length close to 143 cm, seems to be altered compared to other individuals (Fig. 2). This result could indicate a certain level of exposure to some substance with the same characteristics as anticholinesterase. Further study is required in order to better interpret this result, given the high daily mobility of sea lions in their environment, up to 154 km distance from the coast (Hückstädt & Krautz 2004). Hypothetically, sea lions might eat at one contaminated site, located far away from the breeding colony. Around the Nature Sanctuary of Islote Lobería de Cobquecura there is intensive agricultural land-use where pesticides are used, and which via weathering can be transported to the coastal environment and bioaccumulate in the trophic web.

The average concentration of phosphorus found in individuals sampled from the Cobquecura breeding colony (Table 1) was within the same range and is comparable to that reported for other marine mammals (Table 3). The composition of elements in the blood, such as phosphorus, can vary per individual due to diverse factors, such as life stage, sex, and reproductive status (Sterner & Elser 2002). Moreover, phosphorus is present in the structure of important biomolecules, such as nucleic acids (Sterner & Elser 2002) and the skeleton (Diem 1970).

In this study, the differential blood cell counts obtained for *O. flavescens* revealed that most of the circulating white blood cells were polymorphonuclear neutrophils (PMN) and lymphocytes, which is consistent with the normal blood cell count of mammals, including humans (Costa *et al.* 1993). However, the results of the blood cell count reported here also reveal high eosinophilia during the first sampling period, and are significantly different from values during the second period. Values from the first sampling period were also higher than the average percentage of eosinophils observed in Californian sea lions, including clinically healthy and parasite infected animals (Roletto 1993). In the latter study, the range of quantified eosinophils was higher for individuals with parasites (0-12%) compared with those that were clinically healthy (0-8%). Eosinophilia in mammals also has been associated with parasitism (Noemi & Atias 1987, Rodríguez *et al.* 2000, Urquhart *et al.* 2001, Medina *et al.* 2002, Mussart & Coppo 2009). In marine mammals, the existence of skin, lung, stomach, and intestine parasites is widely known (*e.g.*, Carvajal *et al.* 1983, Fernández 1987, Roletto 1993, Dailey *et al.* 2000). For *O. flavescens*, intestinal parasites have been reported (Dailey & Brownell 1972, Cattan *et al.* 1976, George-Nascimento & Carvajal 1981, George-Nascimento & Urrutia 2000), and are acquired during immature developmental stages through ingestion of food (George-Nascimento & Llanos 1995). The high concentration of globulins found during the first sampling period may also be explained by an immune response due to parasitism, which would be consistent with the presence of eosinophilia.

Depending on the type of parasitic tissue, there may be a greater or lesser degree of cell destruction. An indicator of tissue destruction is the enzyme activity of lactate dehydrogenase, or LDH, which is found in the cells of tissues, which upon suffering a lesion; liberate this enzyme into the blood stream (Dufour *et al.* 2005). In this study, LDH displayed low plasma activity, indicating that there was no abnormal tissue damage. In spite of this, eosinophilia detected suggests that parasitism in *Otaria flavescens* may be present. In *O. flavescens*, parasites spend most of their life cycle within the digestive tract (Dailey & Brownell 1972, Cattan *et al.* 1976, George-Nascimento & Carvajal 1981, George-Nascimento & Urrutia 2000). Intestinal parasites, although able to migrate through other organs (*e.g.*, Lungs in the Loos cycle; Steeger & Vargas 1960), produce less damage than parasites that occupy the liver, for example, as their final organ (Carrada-Bravo 2007), or skeletal muscles (Gottstein *et al.* 2009), among others.

Finally, to characterize the health of *Otaria* a larger sample size is needed to conduct a more robust statistical data analysis. The small sample size in this study is insufficient for exploring the complete range of analytes measured in individuals that inhabit the breeding colony. It is nevertheless useful as seminal information for comparing and contrasting with information obtained in the future on the blood chemistry and hematology of this species and therefore, makes a contribution to species conservation in the Southeast Pacific.

Acknowledgments

This study is part of the Programa de Investigación Marina de Excelencia (PIMEX) of the Faculty of Natural and Oceanographic Sciences (Universidad de Concepción, Chile) funded by Celulosa Arauco y Constitución S.A. R. González received additional funding from Programa Copas Sur-Austral (PFB-31). We acknowledge Pablo Carrasco, Danilo Alegría, and Omar Muñoz for their help in the field.

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Received 15 November 2013 and accepted 17 December 2014

Associated Editor: Maritza Sepúlveda M.