



## Spatial, temporal, age, and sex related variation in the diet of South American sea lions in southern Chile

MARITZA SEPÚLVEDA,<sup>1</sup> GUIDO PAVEZ, MACARENA SANTOS-CARVALLO, Centro de Investigación y Gestión de los Recursos Naturales (CIGREN), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Gran Bretaña 1111, Playa Ancha, Valparaíso, Chile and Centro de Investigación Eutropia, Ahumada 131 Oficina 912, Santiago, Chile; CONSTANZA BALBONTÍN, Centro de Investigación y Gestión de los Recursos Naturales (CIGREN), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Gran Bretaña 1111, Playa Ancha, Valparaíso, Chile; GERMÁN PEQUEÑO, Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, casilla 567, Valdivia, Chile; SETH D. NEWSOME, Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, U.S.A.

### ABSTRACT

We analyzed spatial, temporal, and age/sex variation in the diet of the South American sea lions (SASL, *Otaria byronia*) in southern Chile via  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope analysis, with emphasis on exotic farmed and feral salmonids as a source of prey for this generalist predator. Isotope mixing models show that the primary prey consumed by SASL were demersal and benthopelagic fishes. Individuals sampled in summer and in the outer coast had higher  $\delta^{15}\text{N}$  values than those from winter or from the inner coast due to consumption of high trophic level demersal fish. We also found evidence of an ontogenetic shift in isotopic niche width as measured by  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  standard ellipse areas, with juveniles showing a restricted isotopic niche width ( $1.0\text{‰}^2$ ) in comparison to subadult ( $2.2\text{‰}^2$ ) and adult males ( $1.9\text{‰}^2$ ). The proportion of salmonids in the diet of the SASL was negligible and lower than reported in previous studies, which may be related to a recent drop in the production of salmon aquaculture in the region. Our study suggests that the SASL are able to adapt to shifts in spatiotemporal variations in the abundance of potential prey, including feral and farmed salmonids.

Key words: stable isotopes, salmon farming, mixing models, spatial metrics.

In the marine environment, spatial and temporal changes in prey distribution and abundance are expected to result in shifts in the diet composition of generalist and opportunistic marine top predators such as pinnipeds (Guinet *et al.* 2001). For example, the South American sea lion (SASL, *Otaria byronia*) is considered a generalist predator (Cappozzo and Perrin 2009) with a diet primarily composed of fish and, to a lesser degree, mollusks, and crustaceans (Alonso *et al.* 2000). Due to insufficient data it is assumed that the diet of this species remains constant throughout the year and is similar over broad geographic areas and between different age/sex classes. Studies of other closely related otariid species have indicated considerable geographic and

<sup>1</sup>Corresponding author (e-mail: maritza.sepulveda@uv.cl).

seasonal variability in diet over a relatively fine spatial and temporal scale (*e.g.*, García-Rodríguez and Aurióles-Gamboa 2004, McKenzie and Wynne 2008); however, such fine-scale dietary studies are lacking for the SASL.

In Chile, understanding potential variation in diet composition of SASL is relevant because this species strongly interacts with a variety of fisheries and aquaculture operations (*e.g.*, Hückstädt and Antezana 2003, Sepúlveda and Oliva 2005, Goetz *et al.* 2008). Specifically, previous studies have identified a negative interaction between salmon farming and the SASL in southern Chile (Sepúlveda and Oliva 2005, Vilata *et al.* 2010). Accordingly, Muñoz *et al.* (2013) found that farmed salmon was one of the main dietary items for SASL in southern Chile before the large-scale outbreak of the infectious salmon anemia virus that resulted in a significant decline in salmon aquaculture in this region. It is unknown whether salmon is still an important diet item for SASL, and if there are spatial, temporal, sex, or age related variation in how often it or other wild fish of economic importance are consumed by sea lions in southern Chile.

A variety of methods have been used to characterize what sea lions eat. Conventional dietary methods used to study pinniped diets such as scat and gut/stomach content analysis offer several practical problems (Barros and Clarke 2009). Stable isotope analysis (SIA) is a robust proxy for characterizing dietary and habitat preferences of marine mammals (Newsome *et al.* 2010a) that complements traditional techniques, and can be a more reliable way to infer temporal and/or spatial variation in resource use at both the population and individual level (Newsome *et al.* 2009, Hückstädt *et al.* 2012). SIA is particularly useful for detecting temporal variation in diet because different tissues record ecological information over a variety of time scales from weeks (*e.g.*, blood plasma) to months (*e.g.*, red blood cells) and years (*e.g.*, bone collagen) (Martínez del Río *et al.* 2009). Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope values of a consumer reflect those of its diet, with a selective retention of the heavier isotope ( $^{13}\text{C}$  or  $^{15}\text{N}$ ) and excretion of the lighter one ( $^{12}\text{C}$  or  $^{14}\text{N}$ ) (Das *et al.* 2003). This selective retention/excretion yields a somewhat predictable isotopic difference between consumer and prey, also known as trophic discrimination (Karasov and Martínez del Río 2007).  $\delta^{15}\text{N}$  values increase by  $\sim 3\text{‰}$ – $5\text{‰}$  per trophic step and are typically used to quantify trophic level, while  $\delta^{13}\text{C}$  values generally increase by smaller amounts ( $\sim 1\text{‰}$ – $2\text{‰}$ ) per trophic step and are generally used by marine mammal ecologists to assess habitat use (Hobson 1999, Newsome *et al.* 2010a).

The earliest ecological applications of stable isotope values relied on qualitative assessment of data of  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  bivariate plots (or biplots). During the last decade, however, more quantitative measures of trophic position and diet composition have been developed, such as mixing models (Moore and Semmens 2008, Phillips *et al.* 2014) and spatial metrics (Layman *et al.* 2007, Jackson *et al.* 2011). The application of trophic discrimination to consumer tissue isotope values allows for a quantitative estimate of diet through the use of mixing models, which allow researchers to estimate the proportional contribution of sources (dietary items) within a mixture (consumer tissue), and thereby infer consumer diet composition (Phillips *et al.* 2014). Additionally, stable isotope analysis may be used to quantify population or individual level trophic niche width (Layman *et al.* 2007, Jackson *et al.* 2011), which has also been applied to study ecosystem responses to the introduction of exotic species (Olson *et al.* 2009, Sepúlveda *et al.* 2015).

Here we present carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope values from SASL skin samples collected in southern Chile during the summer and winter months from both the inner and outer coasts and for different age/sex classes. We use these data to

quantify diet composition at the individual level using a Bayesian stable isotope mixing model (SIAR; Parnell *et al.* 2008) and to evaluate population-level dietary variation using spatial metrics (Jackson *et al.* 2011). Our goal was to characterize spatial, temporal, and age/sex class related variation in the diet composition of the SASL in this region, with particular emphasis on quantifying the consumption of farmed and feral salmonids.

## MATERIAL AND METHODS

### *Study Area*

The field work was carried out in the Los Lagos region of southern Chile (41°33'S to 43°27'S), characterized by the presence of >300 salmon farming installations, all of them located in the inner or protected coast of this region (Fig. 1). Also, a recent census found that 46 sea lion breeding and haul-out colonies occur in this area on both the inner (protected) and outer (exposed) coasts with an estimated total population of ~44,000 sea lions, accounts for approximately 30% of the total population of this species in Chile (Oliva *et al.* 2012). Sea lion skin samples were collected from six colonies, four on the inner coast (La Arena, Isla Pelada, Cahuelmó, and Chullec) and two on the outer coast (La Sebastiana and Metalqui) (Fig. 1).

### *Stable Isotope Analysis*

Skin samples from 198 sea lions were collected during the austral summer (20 February to 7 March,  $n = 101$ ) and winter (24 July to 07 August,  $n = 97$ ) in 2012. Skin samples reflect dietary information during the same seasons in which they were collected because skin tissue integrates dietary inputs 1–2 mo prior to collection (Kurlle and Worthy 2001). Samples were remotely obtained using a hollow-tipped biopsy dart fired from a PaxArms .22 caliber rifle that produces a small sample of skin, hair, and adipose tissue from SASL without disturbing them. We recorded the date, sample site, and age/sex class of the individual for each sample event. We considered four age/sex classes: (1) juveniles (both males and females), (2) subadult males, (3) adult males, and (4) adult females, according to differences in size, body shape, coloration, and in the case of males the presence of a developed mane (King 1983). The number of samples by season (summer *vs.* winter), geographical zone (inner *vs.* outer), and age/sex class is shown in Table 1.

Samples were stored in sterile plastic 2.0 mL microcentrifuge tubes and frozen for transportation to the laboratory. In the laboratory, samples were manually separated into skin, hair, and adipose tissue. Skin samples were put in heat-sealed Ankom filter bags and dried in an oven (model UNE400, Memmert, Germany) at 60°C for 72 h. Skin samples were lipid-extracted with petroleum ether in a Soxhlet extractor for 2h.

One hundred and thirty samples of 16 potential prey species were also collected from both the inner and outer coasts (Table 2). Fish and crustaceans were collected directly from artisanal fishery landings, including feral salmonids ( $n = 12$ ), while farmed salmonids ( $n = 18$ ) were obtained directly from salmon farms. A ~1 cm<sup>3</sup> subsample of muscle was taken from each prey using a scalpel. Samples of the crustacean squat lobster (*Munida gregaria*) were captured with a 320 µm mesh zooplankton net. After collection, living organisms were left overnight in filtered seawater to evacuate gut contents. All samples were stored in plastic 2.0 mL microcentrifuge tubes and

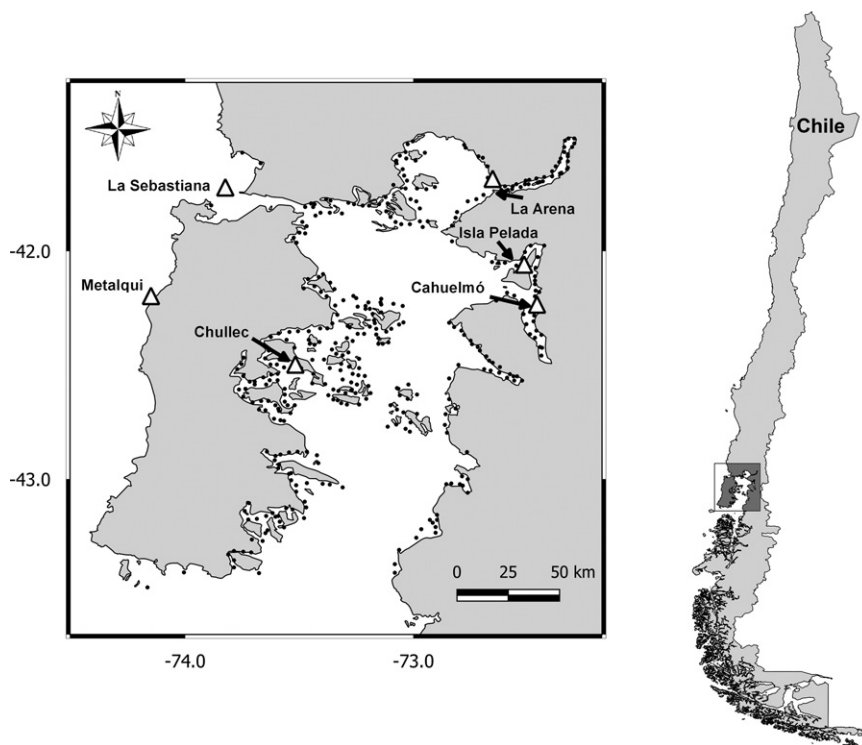


Figure 1. Map of the study area showing the location of the sampled colonies (white triangles) and salmon farms (black circles).

frozen for transportation to the laboratory. In the laboratory, samples were dried in an oven at 60°C for 72 h, homogenized with a mortar and pestle, and the lipid extracted using the same technique described above for sea lion skin. Samples were then stored in 2.0 mL microcentrifuge tubes prior to stable isotope analysis.

Approximately  $0.5 \pm 0.05$  mg of the powdered sample of edible sea lion and prey tissues was sealed in tin capsules and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were measured using a Carlo-Erba NC2500 (Milan, Italy) or Costech 4010 (Valencia, CA) elemental analyzer interfaced with a Finnigan Delta Plus XL mass spectrometer (Waltham, MA) at the University of Wyoming Stable Isotope Facility (Laramie, WY). Isotopic results were expressed as  $\delta$  values,  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = 1,000 \times [(R_{\text{smp}} - R_{\text{std}})/R_{\text{std}}]$ , where  $R_{\text{smp}}$  and  $R_{\text{std}}$  are the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratios of the sample and standard, respectively. The standards were Vienna-Pee Dee Belemnite limestone (VPDB) for carbon and atmospheric  $\text{N}_2$  for nitrogen. The units are expressed as parts per thousand or per mil (‰). Within-run analytical precision (SD) was estimated *via* repeated analysis of internal organic reference materials and measured to be  $<0.2\%$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

We used the Bayesian stable isotope mixing model Stable Isotope Analysis in R (SIAR; Parnell *et al.* 2008) to quantify the relative contributions of five different prey groups to the diet of individual sea lion. The 16 potential prey species were lumped into five distinct groups (Table 2) based on both similarity in their isotopic composition and ecological characteristics; groups included pelagic fishes, demersal fishes,

Table 1. Mean ( $\pm$  SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of SASL skin according to season (summer *vs.* winter), zone (inner *vs.* outer coast), and age/sex class (adult males *vs.* adult females *vs.* subadult males *vs.* juveniles);  $n$  = sample size.

Season	Zone	Age/sex class	$n$	$\delta^{13}\text{C} \pm \text{SD}$	$\delta^{15}\text{N} \pm \text{SD}$
Summer	Inner	Adult male	21	$-13.2 \pm 0.5$	$21.1 \pm 0.6$
		Adult female	16	$-13.6 \pm 0.6$	$20.7 \pm 0.7$
		Subadult male	13	$-13.7 \pm 0.4$	$20.6 \pm 1.0$
		Juvenile	8	$-12.9 \pm 0.5$	$20.6 \pm 0.7$
	Outer	Adult male	5	$-13.6 \pm 0.4$	$21.0 \pm 0.4$
		Adult female	27	$-13.2 \pm 0.4$	$21.3 \pm 0.6$
		Subadult male	4	$-13.6 \pm 0.8$	$21.1 \pm 1.2$
Winter	Inner	Juvenile	7	$-13.3 \pm 0.3$	$20.9 \pm 0.4$
		Adult male	11	$-13.4 \pm 1.0$	$20.0 \pm 1.1$
		Adult female	41	$-13.4 \pm 0.4$	$20.4 \pm 0.6$
		Subadult male	5	$-13.5 \pm 1.1$	$20.0 \pm 1.6$
	Outer	Juvenile	4	$-12.6 \pm 0.3$	$19.4 \pm 0.6$
		Adult male	4	$-14.1 \pm 0.6$	$20.6 \pm 0.9$
		Adult female	27	$-13.5 \pm 0.3$	$20.3 \pm 0.7$
		Subadult male	3	$-13.0 \pm 0.2$	$20.4 \pm 1.2$
		Juvenile	2	$-13.4 \pm 0.1$	$20.6 \pm 0.1$

Table 2. Mean ( $\pm$  SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of each prey species and the five prey groups used in mixing models to quantify SASL diet composition. Zone: (I) inner, (O) outer coast.

Group name	Species	Zone	$n$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
Pelagic crustaceans (PC)	<i>Munida gregaria</i>	I	2	$-19.6 \pm 1.2$	$9.4 \pm 0.5$	
	<i>Oncorbynchus mykiss</i>	I	17	$-17.9 \pm 0.9$	$12.9 \pm 1.4$	
Salmonids (SA)	<i>O. kisutch</i>	I	5	$-19.5 \pm 0.3$	$11.5 \pm 0.6$	
	<i>Salmo salar</i>	I	8	$-19.5 \pm 0.5$	$11.7 \pm 0.5$	
	Mean		30	$-18.6 \pm 1.1$	$12.4 \pm 1.3$	
	Benthopelagic fish (BPF)	<i>Eleginops maclovinus</i>	I, O	12	$-16.7 \pm 2.5$	$15.3 \pm 0.8$
		<i>Mustelus mento</i>	I	2	$-15.2 \pm 0.2$	$16.6 \pm 0.6$
<i>Thyrstites atun</i>		I, O	7	$-16.1 \pm 0.3$	$17.4 \pm 0.9$	
<i>Paralabrax humeralis</i>		I	3	$-12.6 \pm 0.8$	$17.2 \pm 0.1$	
Mean		24	$-15.9 \pm 2.2$	$16.3 \pm 1.2$		
Pelagic fish (PF)	<i>Odontesthes regia</i>	I, O	16	$-16.0 \pm 1.5$	$15.8 \pm 0.7$	
	<i>Sprattus fueguensis</i>	I	7	$-16.4 \pm 0.3$	$15.0 \pm 0.2$	
	<i>Trachurus murphyi</i>	I	4	$-15.4 \pm 0.4$	$17.1 \pm 0.4$	
	Mean		27	$-16.0 \pm 1.2$	$15.8 \pm 0.9$	
Demersal fish (DF)	<i>Merluccius australis</i>	I, O	7	$-15.3 \pm 0.4$	$17.2 \pm 1.0$	
	<i>Cilus gilberti</i>	I, O	3	$-15.4 \pm 0.2$	$17.7 \pm 0.1$	
	<i>Genypterus</i> spp.	I	11	$-15.0 \pm 1.0$	$17.0 \pm 1.1$	
	<i>Callorhynchus callorhynchus</i>	O	3	$-14.3 \pm 0.4$	$18.0 \pm 0.1$	
	<i>Pinguipes chilensis</i>	I	5	$-14.3 \pm 0.6$	$16.8 \pm 0.4$	
	Mean		49	$-15.1 \pm 0.7$	$17.2 \pm 0.9$	

benthopelagic fishes, pelagic crustaceans, and salmonids. Inputs into the model include the mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of each prey group and tissue-specific trophic discrimination factors (TDF) to estimate the probability distributions of the proportional contribution of each prey item (source) in the predator diet (mixture).

Additionally, this model incorporates sources of uncertainty in both prey isotope values and trophic discrimination factors as standard deviation (SD).

At present, there are no published estimates of  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  TDFs for the SASL. We used skin-diet TDF values of  $2.5\text{‰} \pm 0.5\text{‰}$  for  $\delta^{13}\text{C}$  and  $3.0\text{‰} \pm 0.5\text{‰}$  for  $\delta^{15}\text{N}$  in the mixing model. These skin-diet TDFs are slightly higher than estimates for captive phocids reported by Hobson *et al.* (1996), but on par with other mammalian carnivores. The variance estimates (SD) in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  TDFs are based on interindividual variation found in both captive and field-based studies of other marine carnivores (Newsome *et al.* 2010b, Tyrrell *et al.* 2013). We obtained the median contribution of the five prey groups according to season, zone, and age/sex class. Results for the posterior contributions of prey groups to diet are expressed as a mean value ( $\pm$  SD).

### *Spatial Metrics of Trophic Diversity*

We employed three niche metrics to quantify the isotopic niche width of SASL collected in each season, geographical zone, and age/sex class: carbon range (CR), nitrogen range (NR), and standard ellipse areas ( $\text{SEA}_c$ ), and isotopic niche overlap among groups were calculated using the Stable Isotope Bayesian Ellipses in R (SIBER) package in SIAR (Jackson *et al.* 2011). CR and NR were calculated as the distance between the highest and lowest  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values of each group, respectively.  $\text{SEA}_c$  (expressed in  $\text{‰}^2$ ) represents the mean core area of each species' isotopic niche width (Jackson *et al.* 2011); the subscript "c" indicates that a small sample size correction was used when calculating standard ellipse areas (Jackson and Britton 2014). We also calculated the Bayesian estimate of SEA ( $\text{SEA}_B$ ) to compare the size of the ellipses among groups. Both  $\text{SEA}_c$  and  $\text{SEA}_B$  are unbiased with respect to sample sizes  $\geq 15$  (Jackson *et al.* 2011). We also calculated the proportional overlap in  $\text{SEA}_c$  between the different SASL groups, which are reported in units of  $\text{‰}^2$  in Table 1.

### *Data Analysis*

Stable isotope data were transformed with a Box-Cox transformation to satisfy normality conditions. We then compared variation in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  composition of skin collected in winter *vs.* summer, between the inner and outer coast, and among the age/sex classes using a factorial analysis of variance (ANOVA). Multiple comparisons were analyzed using a Tukey HSD *post hoc* test. To test whether the isotopic niche width of the SASL differs between seasons, zones, and/or age/sex classes, we calculated the proportion of estimated posterior ellipses that differ in size between groups (*e.g.*, inner *vs.* outer coast) for each factor. General statistical tests and niche metrics were calculated in R (R Development Core Team 2011). Significance level was set at 95% for all statistical tests. Results are reported as mean ( $\pm$  SD) unless otherwise noted.

## RESULTS

### *Stable Isotope Values*

After correcting for trophic discrimination,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for SASL skin fell well inside the polygon defined by the five prey groups (Fig. 2). Feral and farmed salmonids were placed in the same prey group due to similarity in both  $\delta^{13}\text{C}$  ( $F_{1,31} =$

2.21,  $P = 0.15$ ) and  $\delta^{15}\text{N}$  ( $F_{1,31} = 1.64$ ,  $P = 0.21$ ) isotope values. The overall mean SASL skin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were  $-13.4\text{‰} \pm 0.6\text{‰}$  (range:  $-15.7\text{‰}$  to  $-12.1\text{‰}$ ) and  $20.6\text{‰} \pm 0.8\text{‰}$  (range:  $16.6\text{‰}$  to  $22.6\text{‰}$ ), respectively.

SASL  $\delta^{15}\text{N}$  values were significantly related to season and zone (Table 3). Tissue samples collected in summer had significantly higher  $\delta^{15}\text{N}$  values than tissues collected during the winter. Similarly, samples from the outer coastal zone had significantly higher  $\delta^{15}\text{N}$  values than samples from the inner coastal zone. We found no significant differences in  $\delta^{15}\text{N}$  values among age/sex classes, or for the interactions between factors. On the other hand,  $\delta^{13}\text{C}$  values varied significantly among age/sex classes (Table 3). *Post hoc* analysis revealed that juveniles had significantly higher skin  $\delta^{13}\text{C}$  values than adult females, adult males, and subadult males.  $\delta^{13}\text{C}$  values also show significant differences in the interaction between zone and age/sex classes, in which juveniles from the inner coast had significantly higher  $\delta^{13}\text{C}$  values than adult males and females from the outer coast, and than adult females and subadult males from the inner coast.

### Spatial Metrics

CR and NR results show that the isotopic niche width was broader for SASL in the winter *vs.* summer months, in the inner *vs.* outer coast, and for subadult and adult

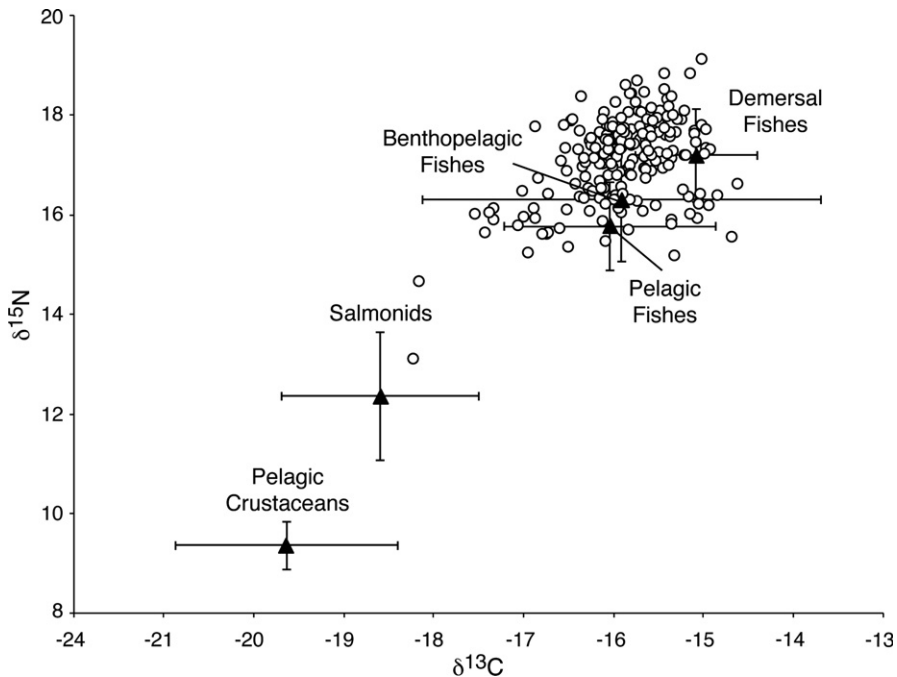


Figure 2.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  biplot showing South American sea lion skin (white circles) samples and the mean ( $\pm$ SD) isotope values of the five prey groups (black triangles) used in mixing models to quantify SASL diet composition. SASL skin isotope values were corrected for trophic discrimination by subtracting  $2.5\text{‰}$  and  $3.0\text{‰}$  from measured  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively.

Table 3. Relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and season, zone, and age/sex class for SASL. Significant differences found with a factorial analysis of variance (ANOVA) are marked in bold.

Factor	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Season	0.05	0.820	19.33	<0.0001
Zone	3.01	0.084	7.20	<b>0.008</b>
Age/sex class	3.60	<b>0.015</b>	0.94	0.423
Season * Zone	0.71	0.401	0.39	0.536
Season * Age/sex class	1.99	0.117	0.05	0.985
Zone * Age/sex class	5.28	<b>0.002</b>	0.49	0.693
Season * Zone * Age/sex class	0.62	0.604	1.96	0.121

males relative to adult females and juveniles (Table 4). SASL  $\text{SEA}_c$  was also significantly higher in winter than summer ( $P = 0.031$ ) (Fig. 3A) and in the inner than outer coast ( $P < 0.001$ ) (Fig. 3B). Furthermore,  $\text{SEA}_c$  was significantly higher for adult males than adult females ( $P < 0.001$ ), subadult males than adult females ( $P < 0.001$ ), and subadult males than juveniles ( $P = 0.03$ ) (Fig. 3C). In terms of isotopic niche overlap, the 95% credible intervals (CI) of Bayesian ellipse areas indicated a 72% probability of overlap between SASL from the inner and outer coastal zones; but only 28% probability of overlap between SASL sampled in the summer and winter months. In regards to age/sex class, a high degree of isotopic niche overlap was observed between adult females and adult males (100%), adult females and subadult males (99%), and between adult males and subadult males (77%), but relatively little overlap was observed between adult females and juveniles (21%), adult males and juveniles (40%), and subadult males and juveniles (18%).

#### Diet Composition

Mixing models showed that the main prey group consumed by SASL was demersal fish, followed by benthopelagic fish (Fig. 4A). The consumption of demersal fishes was similar in both summer and winter seasons. However, benthopelagic fishes were

Table 4. Mean ( $\pm$  SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and isotopic niche metrics of SASL skin grouped by season (summer *vs.* winter), zone (inner *vs.* outer coast), and age/sex class (adult males *vs.* adult females *vs.* subadult males *vs.* juveniles). CR: carbon range, NR: nitrogen range,  $\text{SEA}_c$ : mean ( $\pm$  SD) standard ellipse area ( $\%_00^2$ ).

Factor		<i>n</i>	$\delta^{13}\text{C} \pm \text{SD}$	$\delta^{15}\text{N} \pm \text{SD}$	CR	NR	$\text{SEA}_c$
Season	Summer	101	$-13.4 \pm 0.5$	$21.0 \pm 0.7$	2.6	3.5	1.1
	Winter	97	$-13.4 \pm 0.6$	$20.3 \pm 0.9$	3.6	5.0	1.5
Zone	Inner	119	$-13.4 \pm 0.6$	$20.5 \pm 0.9$	3.6	5.6	1.6
	Outer	79	$-13.4 \pm 0.5$	$20.8 \pm 0.8$	2.5	3.6	0.9
Age/sex class	Adult male	41	$-13.4 \pm 0.7$	$20.7 \pm 0.9$	3.6	3.7	1.9
	Adult female	111	$-13.4 \pm 0.4$	$20.6 \pm 0.7$	2.4	3.6	0.9
	Subadult male	25	$-13.6 \pm 0.8$	$20.5 \pm 1.2$	3.1	5.6	2.2
	Juvenile	21	$-13.0 \pm 0.5$	$20.4 \pm 0.8$	1.6	2.7	1.0



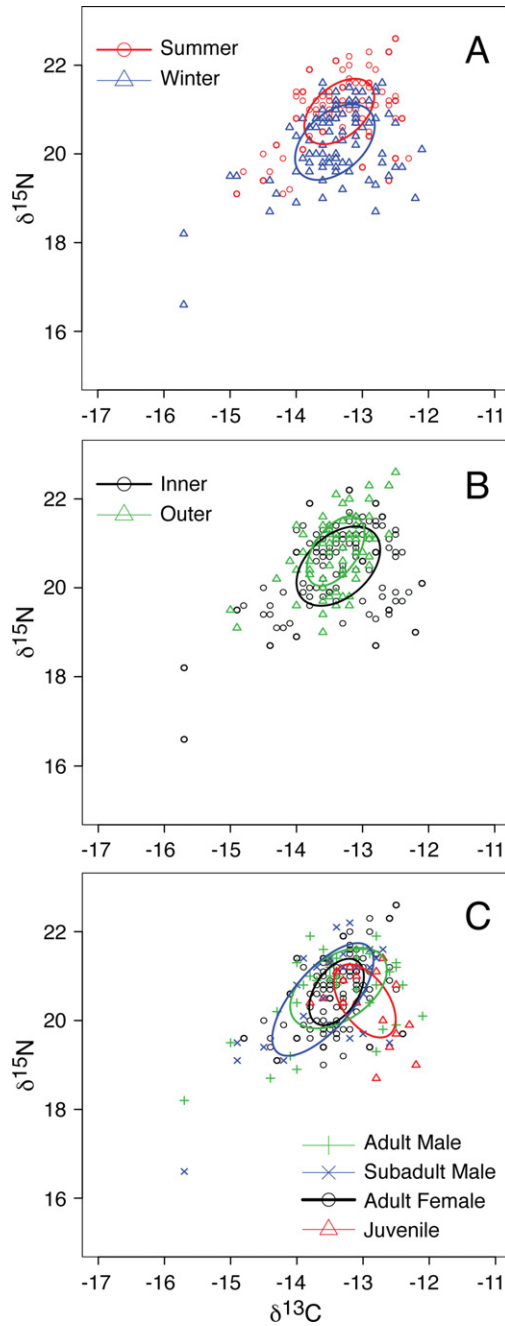


Figure 3.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  standard ellipse areas ( $\text{SEA}_C$  in  $\text{‰}^2$ ) for SASL skin according to (A) season (summer *vs.* winter), (B) zone (inner *vs.* outer coast), and (C) age/sex class (adult males *vs.* adult females *vs.* subadult males *vs.* juveniles).

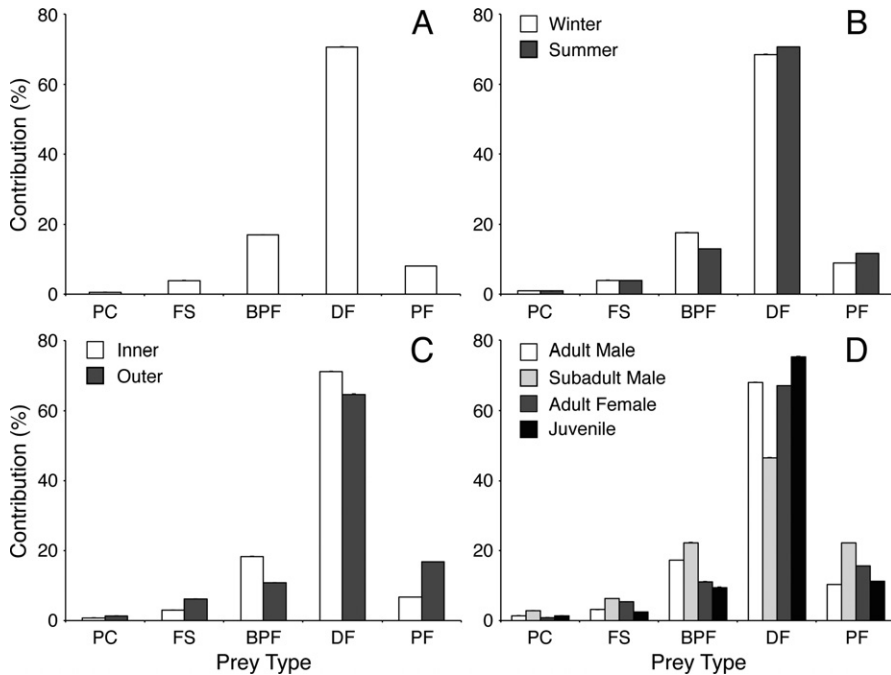


Figure 4. Mean contributions of five prey groups to the diet of SASL grouped by (A) all individuals, (B) season (summer *vs.* winter), (C) zone (inner *vs.* outer coast), and (D) age/sex class (adult males *vs.* adult females *vs.* subadult males *vs.* juveniles). PC: pelagic crustaceans; FS: farmed salmonids; BPF: benthopelagic fish; DF: demersal fish; PF: pelagic fish.

consumed in greater proportion in winter than in summer (Fig. 4B). Both demersal and benthopelagic fishes were consumed in a higher proportion in the inner coastal zone in comparison to the outer zone (Fig. 4C). Contrarily, pelagic fishes were consumed in higher proportion in the outer coastal zone relative to the inner coastal zone. Demersal fishes were consumed in a higher proportion by juveniles in comparison to the other age/sex classes (Fig. 4D). Lastly, the consumption of salmonids was generally low independent of the factor analyzed.

## DISCUSSION

The objective of this study was to determine the diet composition of SASL in the Los Lagos Region of southern Chile and its variability in time, space, and age/sex classes, focusing in particular on estimating the importance of farmed and feral salmonids in their diet. In general, stable isotope mixing models showed that the primary prey consumed by SASL in the study area were demersal and benthopelagic fishes. Similar results were obtained by Muñoz *et al.* (2013), who reported that demersal species such as *M. australis*, *Mustelus mento*, *Genypterus* spp., and *Callorhynchus callosibynchus* are important prey for SASL in the study area. These results are also consistent with previous studies focused on SASL diving behavior along the Atlantic coast, which indicates it is a benthic forager (Thompson *et al.* 1998, Campagna *et al.*

2001, Riet-Sapriza *et al.* 2013, Rodríguez *et al.* 2013, Baylis *et al.* 2015). Along the Pacific coast, recent studies using time-depth recorders and satellite tags also indicated that SASL from southern Chile displayed a mesopelagic-benthic diving strategy (Sepúlveda *et al.* 2015, Hückstädt *et al.* 2016).

SASL skin collected in the summer months had higher  $\delta^{15}\text{N}$  values than skin collected during the winter, a pattern likely driven by seasonal shifts in diet composition. Specifically, mixing models show that SASL primarily consumed demersal fish during the summer, while in winter they consumed a higher proportion of benthopelagic prey. Furthermore, an increase in the consumption of the pelagic fish *S. fueguensis* during the summer is likely related to increased abundance of this prey type in southern Chile during this season (Mujica *et al.* 2013). Lastly, mixing models show that salmonids were consumed in low proportion throughout the year.

SASL from outer coastal zone had higher  $\delta^{15}\text{N}$  values in comparison to their counterparts from the inner coastal zone. According to the mixing model, SASL consumed a higher proportion of demersal and benthopelagic prey in the inner coastal zone, whereas in the outer zone they consumed a higher proportion of pelagic fishes. The inner coastal zone is considered to be one of the most extensive estuarine regions in the world (Iriarte *et al.* 2010) that harbors high concentrations of eggs, larvae, and juvenile life stages of some of the most important demersal and benthopelagic fishes, such as southern hake (*Merluccius australis*) and Patagonian grenadier (*Macruronus magellanicus*) (Bustos *et al.* 2007, Niklitschek *et al.* 2014). Thus, higher availability of demersal and benthopelagic prey could explain the observed spatial variation in SASL diet composition in the inner *vs.* outer coastal zone. It is important to note, however, that differences in  $\delta^{15}\text{N}$  values between outer and inner coastal zones may also be driven by regional patterns in the isotopic composition of the SASL prey (Hirons *et al.* 2001). Pavez *et al.* (unpublished data) found that some species of prey collected in the inner coast had lower  $\delta^{15}\text{N}$  values than those collected on the outer coast. For example, the demersal fish *M. australis* had lower  $\delta^{15}\text{N}$  values in the inner ( $16.7\text{‰} \pm 0.8\text{‰}$ ) *vs.* outer ( $17.6\text{‰} \pm 0.2\text{‰}$ ) coast; the pelagic fish *O. regia* also had lower values in the inner ( $15.3\text{‰} \pm 0.7\text{‰}$ ) *vs.* outer ( $16.3\text{‰} \pm 0.8\text{‰}$ ) coast. Lower  $\delta^{15}\text{N}$  values in the inner coast could result from the influence of nutrients derived from salmon cultivation in the form of unconsumed pellet food and salmon feces (Buschmann *et al.* 2009). The pellet food contains a high percentage of terrestrial soy (*Glycine max*) (Hardy and Castro 1994), and thus has lower  $\delta^{15}\text{N}$  (and  $\delta^{13}\text{C}$ ) in comparison to near-shore marine ecosystems (Karasov and Martínez del Río 2007). These nutrients may alter the isotopic composition of the local benthic and pelagic food webs in two general ways: (1) pellets that are consumed by primary consumers as particulate organic matter (POM) (Vizzini and Mazzola 2004) and/or (2) the excretion of  $^{15}\text{N}$ -depleted ammonia ( $\text{NH}_3$ ) by salmon, which is assimilated directly by micro- and macroalgae (Cohen and Fong 2004). Either scenario would change the isotopic composition of higher trophic levels *via* a bottom-up effect. Thus, the higher  $\delta^{15}\text{N}$  values for sea lions inhabiting the outer coast do not solely indicate consumption of higher trophic level prey, but they may also reflect the geographic baseline differences in the isotope composition of the base of the food web.

Our results show that adult males, subadult males, and adult females had similar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, which suggest a similar diet for these age/sex classes. This is consistent with other isotope-based studies of SASL in Uruguay (Franco-Trecu *et al.* 2014), California sea lions (*Zalophus californianus*) in southern California (Orr *et al.* 2012), and South American fur seals (*Arctocephalus australis*) (Franco-Trecu *et al.* 2014) that reported no differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between the sexes. Our

results show that subadult and adult males, however, had a larger isotopic niche width than females. This pattern may be related to (1) body size as males are larger and thus can feed on a larger range of prey (Costa and Shaffer 2012), and/or (2) differences in the foraging range of male and female SASL. Otariid males are usually present at breeding colonies only during the breeding period (2–3 mo), and consequently may disperse far from the colonies during most of the year and forage over a larger geographic area (Kernaléguen *et al.* 2012). In contrast, adult females with pups, as income breeders, alternate foraging trips at sea and suckling bouts ashore during the whole lactation period (7–12 mo) and are thus confined to a more limited foraging range in close proximity to the colony (Muñoz *et al.* 2011).

Juveniles had a smaller isotopic niche than adults, a pattern that could be driven by differences in experience and physiological capabilities between these age classes (Fowler *et al.* 2007). Juveniles are unable to travel as far or dive as deep as adults, which may restrict them to feeding in shallow coastal areas. This would narrow the choice of suitable prey, and result in differences in the resources and foraging habitats used by juveniles and adults (Jeglinski *et al.* 2012). Also, juveniles had higher  $\delta^{13}\text{C}$  values with respect to the other age/sex classes. According to Jeglinski *et al.* (2012), juvenile Galapagos sea lions (*Zalophus wollebaeki*) spend similar or even longer times at the bottom than adults in shallow waters, a diving pattern that could result in the higher consumption of neritic demersal species by juvenile SASL observed in our study.

Our results indicate that the consumption of farmed salmonids by SASL was lower in 2012 in comparison to what was found by Muñoz *et al.* (2013) in 2009–2010 in the same geographical area. A potential explanation for this pattern is the significant decrease in salmon production in the region in response to a large-scale outbreak of the infectious salmon anemia virus that occurred in 2010 (ISA, Asche *et al.* 2010). Sepúlveda *et al.* (2015) showed that SASL, which previously consumed a high proportion of salmon (Muñoz *et al.* 2013), changed their diet during this period, likely in response to the decrease in salmon availability. In response to the ISA viral outbreak, the salmon industry instigated improvements in management procedures and now there is a much lower density of floating salmon pens in our study area (Niklitschek *et al.* 2013).

Our results suggest that SASL are capable of quickly modifying their diet in response to rapid shifts in prey availability, as has been shown for this species elsewhere and for other otariids (*e.g.*, Cappozzo and Perrin 2009, Sigler *et al.* 2009, Muñoz *et al.* 2013). Since salmon cultivation is rapidly expanding into regions south of our study area (Niklitschek *et al.* 2013), it is imperative to understand how the presence of this novel food source will impact the ecology of SASL, as well as the potential indirect effects (*e.g.*, increased nutrient loads) on nearshore benthic and pelagic food webs. This information is fundamental to understanding the trade-offs between the potentially negative effects of salmon cultivation on native marine ecosystems and the social and economic benefits provided by this important and lucrative industry in Chile.

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