Dieta y dinámica poblacional del león marino del sur (*Otaria flavescens*) en Patagonia

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Diet and population dynamics of the South American sea lion (*Otaria flavescens*) in Patagonia

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Alguien dijo una vez:

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Contenidos

Introducción General y Objetivos

Los seres humanos dependen de los ecosistemas marinos para la obtención de una serie de importantes y valiosos bienes y servicios, pero el incremento de las actividades humanas, derivadas del crecimiento exponencial de la población humana y del aumento del consumo de energía *per capita*, están generando un notable impacto ecológico a gran escala sobre los ecosistemas marinos (Halpern *et al*., 2008).

La visión de los océanos como fuente inagotable de recursos y su explotación para sustentar las exigencias de la creciente población humana se ha traducido en una rápida alteración de los ecosistemas marinos de todo el mundo (Pauly *et al*., 1998; Jackson & Sala, 2001; Jackson *et al*., 2001; Myers & Worm, 2003). Desde hace casi dos décadas, más de dos tercios de los recursos pesqueros en el planeta se hallan plenamente explotados, sobreexplotados o agotados (Botsford *et al*., 1997). Además, estudios más recientes sugieren que incluso las cifras pueden ser demasiado optimistas ya que la abundancia de las poblaciones marinas en el pasado ha sido a menudo pasada por alto u olvidada (Jackson *et al*., 2001). En concreto el análisis del registro arqueológico, está ofreciendo estimaciones de datos históricos de abundancia que comparados con los de hoy en día no pueden arrojar otra conclusión sino la de que los océanos del planeta se degradan a marchas forzadas (Jackson *et al*., 2001; Lotze & Milewski, 2004). Estos datos son una muestra de hasta que punto la actividad humana ha cambiado la composición biológica de los océanos y la estructura y funcionamiento de su redes tróficas (Springer *et al*., 2003; Emslie & Patterson, 2007). La magnitud de los cambios es tal que, si el sistema alcanzara un nuevo estado estable, podría verse impedida la restauración de su dinámica inicial incluso aunque cesase la explotación humana (Petraitis & Dudgeon, 2004).

En este proceso de degradación, uno de los grupos más afectados es el formado por aquellos animales situados en la parte superior de las redes tróficas, pues combinan poblaciones relativamente pequeñas con bajas tasas de crecimiento demográfico. Entre ellos destacan los pinnípedos, como uno de los taxones animales que más han sufrido el efecto de las actividades humanas (Costa *et al*., 2006; IUCN, 2009; Merrick *et al*., 2009). Varias especies de pinnípedos se han extinguido en las últimas décadas, como la foca monje del Caribe (*Monachus tropicalis*) y el león marino japonés (*Zalophus japonicus*), o

se hallan virtualmente al borde de la extinción, como la foca monje del Mediterráneo (*Monachus monachus*). Otras especies, como por ejemplo el león marino de Steller (*Eumetopias jubatus*) o el león marino australiano (*Neophoca cinerea*), han experimentado severas reducciones de sus poblaciones, hasta el punto de haber sido incluidas en la lista roja de especies amenazadas de la Unión Internacional para la Conservación de la Naturaleza (IUCN). Por ultimo, la mayor parte de aquellas poblaciones que aún son abundantes, como por ejemplo la del león marino de Nueva Zelanda (*Phocarctos hookeri*) o del oso marino del norte (*Callorhinus ursinus*), muestran evidencias de severos impactos negativos que a la larga podrían resultar insostenibles (Costa *et al*., 2006; IUCN, 2009; Merrick *et al*., 2009).

Utilizados desde hace al menos 5000 años por ciertas comunidades humanas costeras, los pinnípedos fueron perseguidos a gran escala, principalmente por el comercio de su piel y grasa, desde finales del siglo XVIII (Bonner, 1982). Al igual que otros mamíferos marinos, también se les persiguió para reducir la competencia por la pesca, ya que en algunos lugares han sido considerados como una plaga que merma los beneficios del sector pesquero (Wickens *et al*., 1992; Barlow, 2009).

Afortunadamente, a partir de los años sesenta, gracias a un cambio de actitud ética global y a mayores presiones conservacionistas internacionales, la explotación comercial ha declinado considerablemente o ha desaparecido, y allí donde todavía existe intenta ajustarse a las capacidades de productividad de las poblaciones afectadas (Barlow, 2009; Reeves, 2009a,b).

A parte de la explotación comercial, que en un intervalo de tiempo relativamente corto ha causado la extinción de algunas especies y la drástica reducción de otras, una de las principales causas de recesión de los pinnípedos, o de la falta de recuperación de sus poblaciones tras el cese de la explotación, es la reducción de la disponibilidad de alimento causada por la sobreexplotación pesquera (Costa *et al*., 2006).

La pesca ha sido identificada como la actividad humana con mayor capacidad de inducir cambios en los ecosistemas marinos, a través de la extinción ecológica de especies clave (Jackson *et al*., 2001). La pesca no sólo amenaza directamente a las poblaciones de pinnípedos a través de su explotación y de las capturas accidentales. Además, representa un factor fundamental en el proceso de reducción de la capacidad de carga de sus hábitats

(Costa *et al*., 2006; Northridge, 2009; Plagányi & Butterworth, 2009) particularmente en mares donde el impacto de la pesca es elevado y las poblaciones de peces o cefalópodos que forman la dieta de los pinnípedos (Barros & Clarke, 2009) han sido fuertemente reducidas. Además, al impacto pesquero se le debe añadir el cambio climático y otros fenómenos ambientales, como El Niño-Oscilación del Sur (ENSO) o la Oscilación Nord Atlántica (NAO), todos ellos susceptibles de actuar de modo aditivo a la pesca, produciendo alteraciones, ya sea periódicas o a largo plazo, en la productividad de las aguas y la consecuente disponibilidad de presas (Trillmich & Ono, 1991; Gerber & Hilborn, 2001; Carretta *et al*., 2002; Johnston *et al*., 2005).

El efecto de estos procesos en la supervivencia de los individuos y, a largo plazo, de las poblaciones, es bien conocido. Se sabe desde hace ya tiempo que la fertilidad, la supervivencia de los neonatos, la calidad de la leche y otras variables que determinan las tasas de reproducción de los pinnípedos se ven severamente afectadas por la disponibilidad de alimento. Lo mismo sucede con la resistencia a las enfermedades y, en general, con la supervivencia (Boyd, 1984, 1996; Guinet *et al*., 1998; Soto *et al*., 2004, 2006; Trites & Donnelly, 2003; Geraci & Lounsbury, 2009). Por lo tanto la disponibilidad de alimento y la condición nutritiva determinan en gran medida la capacidad de respuesta de las poblaciones de pinnípedos frente a factores externos, ya sean ambientales o de origen antropogénico. En concreto, estas variables condicionan la elasticidad y el poder de recuperación de una población después de haber sufrido una reducción en el número de efectivos.

Entre los pinnípedos, los otáridos y aquellos fócidos con una biología similar, como la foca monje del Mediterráneo (*M. monachus*) (Aguilar *et al*., 2007), son los más sensibles a las actividades humanas y por lo tanto se enfrentan a un riesgo de extinción mayor. Sus poblaciones son más vulnerables ya que la larga vinculación a tierra derivada de sus largos periodos de lactancia incrementa la vulnerabilidad a los depredadores, incluido el hombre. Por otro lado, la necesidad de alimentarse durante la lactancia les hace más vulnerables a cambios, antropogénicos o naturales, en la disponibilidad local de alimento (Costa *et al*., 2006; Bowen *et al*., 2009).

Las diferencias antes mencionadas en la estrategia de reproducción y de alimentación de los otáridos con respecto a las de los fócidos, así como una distribución

menos polar, explican las marcadas diferencias en las tasas de recuperación de ambos grupos; así, 15 de las 19 especies de fócidos superan los 100.000 individuos mientras sólo 8 de las 17 especies de otáridos lo hacen (Costa *et al*., 2006; Bowen *et al*., 2009). Un claro ejemplo de estas diferencias puede observarse en la isla de Guadalupe, donde durante el periodo posterior a la explotación comercial la población de elefante marino septentrional (*Mirounga angustirostris*) se ha recuperado mucho mas rápidamente que la de cualquiera de las dos especies de otáridos allí presentes, alcanzando en los años noventa los 15.000 individuos frente a los 11.000 de osos marinos (*Arctocephalus townsendi*) y los pocos menos de un centenar de leones marinos (*Zalophus californianus*) (Costa *et al*., 2006).

Este ejemplo también muestra las diferencias en la respuesta dinámica a una perturbación entre los dos grupos de otáridos, los osos marinos de la subfamilia Arctocephalinae y los leones marinos de la subfamilia Otariinae. En un contexto mundial, el estado de conservación de las poblaciones de osos marinos es menos preocupante que el de los leones marinos, ya que sus poblaciones tienden a incrementar más rápidamente y a superar su número en un orden de magnitud (aproximadamente 9 millones en osos marinos en comparación con poco mas de 700.000 leones marinos, Tabla 1).

Estas diferencias parecen estar relacionadas con las diferencias en el comportamiento de inmersión, y por lo tanto con los hábitos de alimentación, de los dos grupos (Costa & Gales, 2003; Costa *et al*., 2001, 2004, 2006). Los animales con hábitos bentónicos operan cerca del límite fisiológico de buceo aeróbico y gastan mas energía y tiempo en la búsqueda de alimento que las especies de hábitos pelágicos. En estas condiciones, tienen un menor margen para incrementar el esfuerzo de búsqueda de alimento cuando la disponibilidad del mismo disminuye debido al incremento de la explotación pesquera o a cambios ambientales (Costa *et al*., 2004, 2006). Esto explicaría por que la mayor parte de las poblaciones de leones marinos (ej. *Eumetopias jubatus, Neophoca cinerea*, *Phocarctos hookeri*, *Otaria flavescens*) y también de algún oso marino con hábitos bentónicos (ej. *Arctocephalus pusillus doriferus*) (Costa *et al*., 2004, 2006) se hallen en una situación de declive, estática o de lento incremento, que ha llevado a catalogarlas como amenazadas (Tabla 1). Contrariamente, la mayor parte de las poblaciones de osos marinos (ej. *Arctocephalus gazella, Arctocephalus tropicales, Arctocephalus pusillus pusillus*) pero también de algún león marino (ej. *Zalophus*

californianus) con hábitos pelágicos (Costa *et al*., 2004, 2006) están experimentando crecimientos sustanciales y la mayoría están catalogadas como especies de preocupación menor, a pesar de haber sido intensamente explotadas durante los siglos XVIII y XIX (Tabla 1).

Sin embargo, dentro de una misma especie también se observan respuestas diferentes en función de la población. Por ejemplo, en Alaska, la población occidental del león marino de Steller (*E. jubatus*), continúa declinando aun hoy en día, mientras que la población oriental presenta un notable aumento, a pesar de que ambas poblaciones fueron aparentemente explotadas con una intensidad similar (Raum-Suryan *et al*., 2002). Lo mismo pasa con la población de león marino del sur (*Otaria flavescens*) en las islas Malvinas con respecto a aquella de las costa Argentina (Reyes *et al*., 1999; Dans *et al*., 2004; Schiavini *et al*., 2004; Thompson *et al*., 2005).

Tabla 1. Estatus, tendencias y tamaño de las poblaciones de otáridos a nivel global (IUCN, 2009). Definición del estatus según la IUCN: EX (Extinto), EP (En peligro: riesgo muy alto de extinción), VU (Vulnerable: riesgo alto de extinción), CA (Casi amenazado: próximo a satisfacer los criterios de EP ó VU), PM (Preocupación menor: no cumple ninguno de los criterios que definen las categorías de EP, VU o CA).

Nombre científico	Nombre común	Tamaño	Tendencia	Estatus
Hemisferio norte				
Eumetopias jubatus	León marino de Steller	105.800-117.800	Decreciente	EP
Callorhinus ursinus	Oso marino del norte	~1.100.000	Decreciente	VU
Zalophus californianus	León marino de California	~355.000	Creciente	PM
Arctocephalus townsendi	Oso marino de Guadalupe	15.000-17.000	Creciente	CA
Zalophus japonicus	León marino japonés			EX
Zalophus wollebaeki	León marino de Galápagos	20.000	Decreciente	EP
Arctocephalus galapagoensis	Oso marino de Galápagos	10.000-15.000	Decreciente	EP
Hemisferio sur				
Otaria flavescens	León marino del sur	>250.000	Estable	PM
Arctocephalus australis	Oso marino sudamericano	250.000-300.000	Creciente	PM
Arctocephalus philippii	Oso marino de Juan Fernández	>12.000	Creciente	CA
Arctocephalus pusillus pusillus	Oso marino sudafricano	2.000.000	Creciente	PM
Neophoca cinerea	León marino australiano	~13.790	Decreciente	EP
Arctocephalus pusillus doriferus	Oso marino australiano	92.000	Creciente	PM
Arctocephalus forsteri	Oso marino de Nueva Zelanda	~200.000	Creciente	PM
Phocarctos hookeri	León marino de Nueva Zelanda	~11.855	Decreciente	VU
Arctocephalus tropicalis	Oso marino subantártico	>310.000	Creciente	PM
Arctocephalus gazella	Oso marino antártico	4-6 ($\times 10^6$)	Creciente	PM

El león marino del sur

El león marino del sur, también comúnmente llamado león marino sudamericano, lobo marino de un pelo, lobo marino del sur, lobo común, lobo chusco o leâo marinho, en función de la localidad, es una de las siete especies pertenecientes a la subfamilia Otariinae (familia Otariidae; orden Carnivora) y la única perteneciente al género *Otaria* (Riedman, 1990; Cappozzo & Perrin, 2009). Su identidad taxonómica ha resultado motivo de controversia durante largo tiempo, pues dos nombres eran utilizados hasta hace pocos años: *Otaria flavescens* (Shaw, 1800) y *Otaria byronia* (de Blainville, 1820). Finalmente, Rodríguez & Bastida (1993) concluyeron definitivamente que el nombre válido era *Otaria flavescens*. El nombre del género procede del griego *otarion* (oreja pequeña), por sus pequeños pabellones auditivos, mientras el epíteto de la especie deriva del latín *flavus* (amarillento) y hace referencia al color de la cría de león marino del sur proveniente del estrecho de Magallanes que fue empleada por Shaw como holotipo para describir a la especie (Rodríguez & Bastida, 1993).

Esta especie es una de la más grandes y más dimórficas entre las especies de otáridos (Cappozzo & Perrin, 2009). Los machos adultos se distinguen claramente por su gran volumen corporal (aproximadamente 3 metros de longitud y 350 Kg de peso) de las hembras adultas (aproximadamente 2 metros de longitud y 150 Kg de peso), así como por presentar una coloración más oscura y una fisonomía leonina debido al desarrollo de una característica y densa melena (Figura 1). Su marcado dimorfismo sexual se pone de manifiesto desde temprana edad, siendo los machos un 10% más pesados y un 4% más largos que las hembras ya al nacer (Cappozzo *et al*., 1991).

La duración media de la vida de esta especie es de aproximadamente 18-20 años, alcanzando los machos la madurez sexual alrededor de los 6 años de edad y las hembras alrededor de los 4 años de edad (King, 1983; Cappozzo & Perrin, 2009; Grandi *et al*., 2009).

El león marino del sur no es una especie migratoria, manteniéndose en la zona costera durante todo el año, aunque ciertos ejemplares son capaces de realizar desplazamientos de centenares de kilómetros (Riedman, 1990). Se distribuye ampliamente a lo largo de todas las costas sudamericanas, desde Torres (sur de Brasil - 29º20’S,

49º43’W) hasta Cabo de Hornos, incluyendo las Islas Malvinas, en el Atlántico, y desde Cabo de Hornos hasta Zorritos (norte de Perú - 3º40’S, 80º34’W) en el Pacífico (Cappozzo & Perrin, 2009). La población mundial estimada es superior a los 250.000 individuos, con aproximadamente 150.000 individuos a lo largo de la costa pacífica y aproximadamente 120.000 individuos a lo largo de la costa atlántica, principalmente concentrados en Argentina (Cappozzo & Perrin, 2009; IUCN, 2009).

Figura 1. Aspecto externo del león marino del sur, pudiendo observarse el marcado dimorfismo sexual.

De hecho, en Argentina, y en particular a lo largo de la costa patagónica, donde se estima que su población alcanza aproximadamente los 110.000 individuos, se trata del mamífero marino más abundante y más fácil de observar, contando con numerosos apostaderos continentales e insulares que utiliza para reproducirse y/o como lugares de asentamiento invernales (Reyes *et al*., 1999, Dans *et al*., 2004; Schiavini *et al*., 2004).

El sistema de apareamiento es poligínico: un macho adulto defiende y copula con un grupo de cuatro a diez hembras, constituyendo lo que se conoce comúnmente como *harén*. (Campagna & Le Boeuf, 1988). Los machos compiten agresivamente para mantener su supremacía sobre el *harén* hasta el momento de la cópula, defendiéndolo sobre todo de aquellos machos juveniles y subadultos que no forman harenes y suelen congregarse en la periferia de las colonias ejecutando en muchos casos comportamientos “antisociales” como rapto de hembras y crías, que quizá representa una de las causas más importantes de mortalidad de las crías ya que pueden morir por inanición debido a la separación de las

madres o por abusos físicos (Campagna *et al*., 1988a,b; Campagna *et al*., 1992). Los territorios defendidos por los machos son utilizados por las hembras para parir y amamantar a las crías (Campagna & Le Boeuf, 1988).

Los primeros en llegar a los apostaderos son los machos, muchos de los cuales ayunan durante toda la temporada de cría. Luego comienzan a llegar las hembras, que al acabo de un par de días paren y una semana después copulan. A los dos días de copular, las hembras inician los viajes de alimentación al mar. Estos viajes tienen una duración media de tres días y se alternan con estancias en tierra de dos días de duración media, durante las cuales amamantan a las crías (Campagna & Le Boeuf, 1988). Tres o cuatro semanas después del parto las crías entran en agua por primera vez aunque su destete se produce alrededor de un año (Campagna, 1985; Oftedal *et al*., 1987).

La temporada de cría tiene lugar durante el verano austral, iniciándose en toda su área de distribución a mediados del mes de diciembre. A pesar del sincronismo en su inicio, la duración de la temporada parece aumentar al disminuir de la latitud (Campagna, 1985). En particular, en el norte de Patagonia, la máxima densidad en la colonia, tanto de machos como de hembras adultas, se alcanza durante la segunda quincena de enero, período en el que se concentra también el número máximo de cópulas y el mayor porcentaje de nacimientos (Campagna, 1985). En la segunda semana de febrero la actividad reproductiva declina y las áreas de cría comienzan a disolverse. Hacia el final de febrero, la temporada de reproducción puede considerarse acabada ya que no se observan ni nacimientos ni cópulas (Campagna, 1985).

El león marino del sur, al igual que otras especies de su familia, fue intensamente perseguido por su piel y grasa. En las costas sudamericanas, y principalmente en Patagonia, las poblaciones del león marino del sur no escaparon a este contexto histórico mundial. La especie fue objeto de explotación desde comienzos de la colonización europea; sin embargo la mayor explotación del león marino del sur se produjo entre 1920 y 1950 (Godoy, 1963). Se estima que en la década de 1960, cuando cesó la explotación, la población se había reducido a menos del 10% de su tamaño original (Crespo & Pedraza, 1991; Reyes *et al*., 1999; Schiavini *et al*., 2004).

Tras el cese de la explotación, la población norpatagónica permaneció sin crecer hasta la década de 1980. Se inició entonces un ligero crecimiento del 1,3% anual hasta

finales de la década de 1990, cuando se intensificó de un modo muy marcado (Crespo & Pedraza, 1991). Actualmente, la población del león marino del sur en el norte de Patagonia aumenta anualmente en un 5,7% y cuenta con unos 50.000 individuos, cifra que representa aproximadamente un tercio de la población estimada antes de la explotación (Dans *et al*., 2004). La misma tendencia en la dinámica poblacional se ha observado en el sector patagónico centro-meridional (Reyes *et al*., 1999, Schiavini *et al*., 2004).

El crecimiento de la población se traduce en la consolidación de nuevas áreas de cría en la periferia de las ya existentes (Dans *et al*., 2004; Grandi *et al*., 2008). Este fenómeno se observa claramente en el apostadero de Punta León (Figura 2), que junto con los apostaderos de Punta Buenos Aires y Punta Norte, ambos ubicados en la Península Valdés, representan las áreas de cría más importantes en el norte de Patagonia, sumando entre las tres alrededor del 60% de las crías nacidas en el año 2001 (Dans *et al*., 2004). Durante los últimos diez años se observó en Punta León, además del aumento en el número de crías, un aumento del espacio ocupado en la playa (Crespo *et al*., 2002; Dans *et al*.,

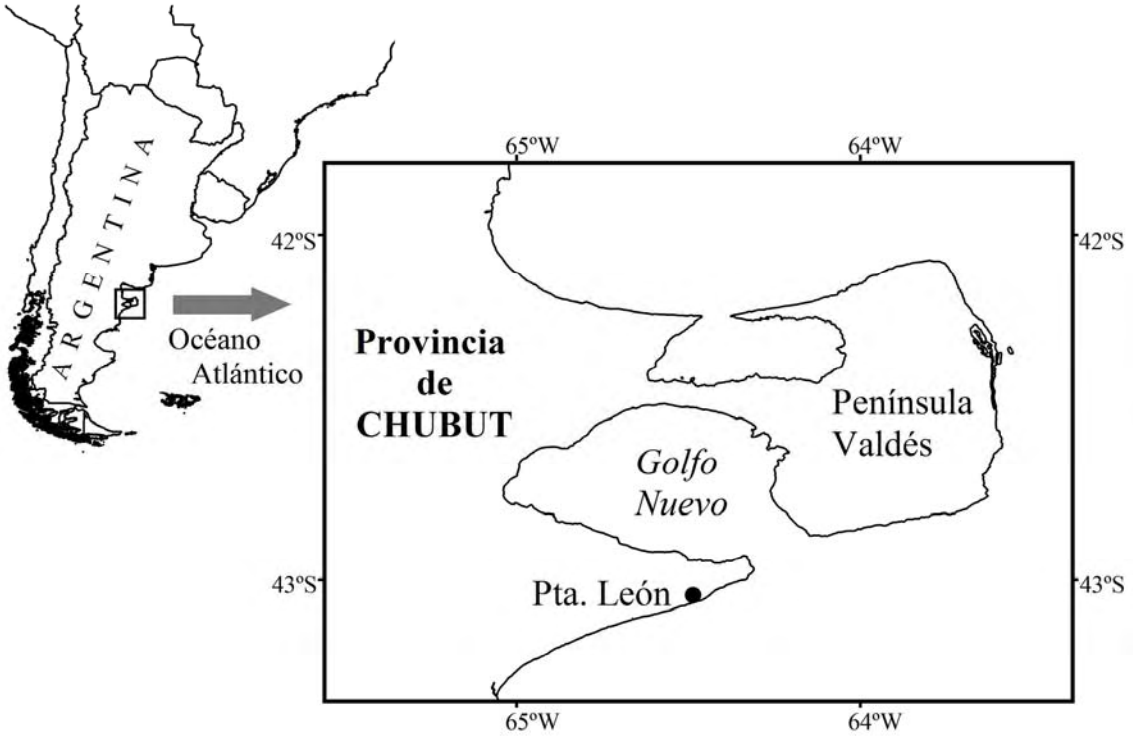

Figure 2. Detalle de la costa de Península Valdés y zona adyacentes, mostrando la ubicación del apostadero de Punta León.

2004), básicamente debido a la aparición de nuevos grupos de cría situados a unos 2-3 Km hacia el suroeste del área principal de cría del apostadero (Crespo *et al*., 2002; Dans *et al*., 2004). Estos pequeños grupos estaban constituidos, en un primer momento, principalmente por juveniles e incluían sólo unas pocas hembras con crías, pero en el transcurso de los años fueron transformándose en áreas de cría (Crespo *et al*., 2002; Grandi *et al*., 2008).

Este proceso ha dado lugar a la existencia de dos tipos de área de cría: las denominadas "tradicionales", que presentan la estructura social típica de grupos reproductivos descrita para la especie (Campagna & Le Boeuf, 1988), y las "de expansión", las cuales corresponden a los nuevos grupos formados dentro de agrupaciones de juveniles. Los grupos de cría nuevos o de expansión presentan características diferentes a las de los grupos de cría tradicionales. Las diferencias más notables se observan en variables tales como el incremento anual en el número de crías, la composición por clases de edad y sexo y la mortalidad de crías durante la temporada reproductiva (Crespo *et al*., 2002). Así, en el sector de cría tradicional se observa una tasa de incremento en el número de crías cercana al 3% anual entre 1983 y 2001, mientras que el sector nuevo presenta una tendencia del 20% entre 1994 (año en que se detectaron los primeros grupos en este sector) y 2001 (Crespo *et al*., 2002). Los grupos de cría nuevos poseen una mayor proporción de juveniles de ambos sexos y mayor proporción de machos subadultos que los grupos tradicionales (Crespo *et al*., 2002). Por último, otra diferencia observada en Punta León entre los dos tipos de áreas de cría es la tasa de mortalidad de crías durante las primeras semanas de vida, de un 2% para el sector tradicional y un 5% para el sector nuevo (Crespo *et al*., 2002).

Todo ello sugiere que la lenta recuperación inicial de la población de león marino del sur tras el cese de la matanza fuera debida a la pérdida de la estructura social. Ahora bien, simultáneamente al cese de la caza del león marino del sur, frente a la provincia de Chubut se inició la pesca industrial de la merluza argentina (*Merluccius hubbsi*) y del langostino (*Pleoticus muelleri*) (Crespo *et al*., 1997; Bertolotti *et al*., 2001). La merluza argentina es una de las presas principales del león marino del sur (Koen Alonso *et al*., 2000), junto con el pulpo colorado (*Enteroctopus megalocyathus*) y el calamar argentino (*Illex argentinus*), motivo por el cual el desarrollo de la pesquería también podría haber afectado a la recuperación de león marino del sur, no sólo por una posible reducción en la

disponibilidad de alimento, sino también por un cambio general en el funcionamiento del ecosistema (Koen Alonso & Yodzis, 2005). De ser esto cierto, cabría esperar cambios en la dieta del león marino del sur a medida que se desarrollaba la pesquería, así como cambios en el tamaño de los adultos en caso de existir una limitación de la cantidad de alimento disponible.

La revisión de los datos históricos sobre el tamaño de la población del león marino del sur y de los desembarques y biomasa de merluza argentina (*M. hubbsi*) (Bertolotti *et al*., 2001; Dans *et al*., 2003; Lloris *et al*., 2003; Koen Alonso & Yodzis, 2005) indica la existencia de cuatro períodos con una diferente disponibilidad *per capita* de merluza para los leones marinos del sur en las costas de la provincia de Chubut (Figura 3). El primer período, sin presión pesquera y con una población del león marino del sur en declive, abarcaría el período de la matanza (1920-1960). El segundo período, caracterizado por una elevada disponibilidad de merluza ya que tanto los desembarques pesqueros como la población de leones marinos se hallaban en sus mínimos históricos, abarcaría las décadas de los años sesenta y setenta (1960-1980). El tercer período, caracterizado por un descenso de la disponibilidad de alimento debido a un continuo incremento de los desembarques de

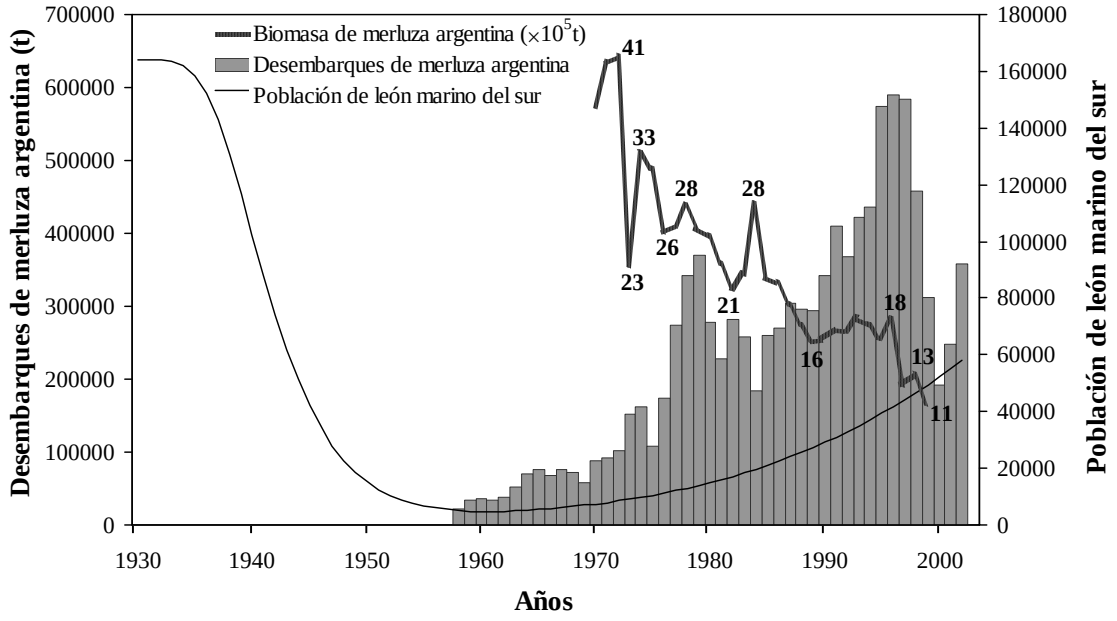

Figura 3. Desembarques y biomasa de merluza argentina y dinámica poblacional del león marino del sur en el norte y centro de la Patagonia (Dans *et al*., 2003; Koen Alonso & Yodzis, 2005)

merluza, abarcaría los años ochenta y parte de los noventa (1981-1997). Finalmente, el último período, caracterizado por un incremento de la población del león marino del sur y el colapso de la pesquería de merluza, comprende los años entre 1998 y la actualidad.

Uso de isótopos estables en ecología trófica

Considerando que la disponibilidad de alimento es un determinante crítico del bienestar de los individuos y en general de la dinámica poblacional de la mayoría de las especies, el conocimiento de la ecología trófica es fundamental para una gestión efectiva de su conservación. En particular el conocimiento exacto de la composición de la dieta, las fuentes de alimento, incluidas aquellas derivadas de las actividades humanas, como por ejemplo los descartes pesqueros, las estrategias y los hábitos alimenticios, la variación espacio-temporal en la ecología alimentaría y las interacciones entre los hábitats explotados y las actividades humanas, se convierten en esenciales para comprender cuando las especies son más vulnerables o cuando las poblaciones tienen más probabilidades de verse limitadas por los alimentos, al fin de facilitar la predicción de cambios de alimentación y sus consecuencias sobre la dinámica de las poblaciones.

Las cuestiones relacionadas con la ecología trófica de las especies marinas se han abordado durante años mediante el análisis del contenido estomacal y de los excrementos. Aunque estos métodos han demostrado poder resolver adecuadamente la estructura de la cadena trófica y proporcionar información taxonómica sobre la dieta, muestran sin embargo limitaciones (Michener & Schell, 1994). Uno de los principales problemas es la identificación de las presas, ya que se suele sobrestimar la contribución a la dieta de presas con partes duras, más resistentes a la digestión, y por el contrario subestimar la contribución de presas blandas, de difícil identificación porque son fácilmente digeribles, y también, como ocurre en algunos pinnípedos, de aquellas presas con partes duras que son regurgitadas (Gales & Cheal 1992; Michener & Schell 1994; Jordan, 2005). Además estas metodologías proporcionan una visión episódica de la dieta del individuo, ya que cada muestra representa sólo un evento específico de alimentación y no proporciona información sobre los recursos utilizados en el pasado (Michener & Schell, 1994; Burns *et al*., 1998). Por lo tanto, para obtener información fiable sobre los hábitos alimenticios a

largo plazo es necesario un seguimiento exhaustivo y sistemático en el tiempo (Jordan, 2005).

En el caso de los pinnípedos, existen problemas específicos derivados de su talla y comportamiento. En primer lugar, el muestreo repetido de animales de gran tamaño para el análisis del contenido estomacal es extremadamente difícil. También es difícil asignar adecuadamente las heces a individuos concretos en colonias abarrotadas. Por ultimo, la mayoría de los estudios basados en métodos convencionales se centran en la dieta durante el período de reproducción, ya que la mayoría de las especies de pinnípedos son difíciles de muestrear fuera de la temporada de cría. Se descuida de esta manera gran parte del año, ignorándose por ejemplo los cambios estacionales (Dalerum & Angerbjörn, 2005).

Uno de los enfoques más difundidos en los últimos años para superar o mitigar las limitaciones antes indicadas es el análisis de isótopos estables y en particular el de los isótopos estables de carbono y nitrógeno (Michener & Schell, 1994; Cuadro 1).

Cuadro 1. ***Isótopos estables***

Se denominan isótopos (del griego *isos* = mismo y *tópos* = lugar) a los diferentes tipos de átomos de un mismo elemento químico con igual número atómico (número de protones en el núcleo) pero diferente masa, debido a diferencias en el número de neutrones. A pesar de ello, sus características físicas y químicas no cambian sustancialmente.

La mayoría de los elementos químicos poseen más de un isótopo; estos se subdividen en isótopos estables y no estables (radiactivos), aunque el concepto de estabilidad no es del todo exacto ya que su estabilidad se debe al hecho que tienen un tiempo de neutralización extremamente largo (Hoefs, 2004).

Hay muchos elementos con múltiples formas isotópicas estables, pero solo aquellos relacionados con la biosfera, la hidrosfera y la atmósfera se utilizan en la investigación ecológica, es decir, los de carbono, nitrógeno, hidrógeno, oxigeno y azufre (West *et al*., 2006).

Comúnmente, los isótopos estables más utilizados para los estudios tróficos de organismos marinos son los del carbono y el nitrógeno (Michener & Schell, 1994; Kelly, 2000). La forma más abundante de carbono es el isótopo estable ligero ^{12}C (98,89%), pero también existe un isótopo estable pesado ^{13}C menos frecuente (1,11%). El nitrógeno también presenta dos formas isotópicas estables, siendo ^{14}N el isótopo más común (99,63%) y ^{15}N el presente en menor proporción (0,37%) (West *et al*., 2006).

Los isótopos estables se cuantifican de diversas maneras, pero el método más común es mediante un espectrómetro de masa de relación isotópica, que proporciona con gran exactitud la estimación de la proporción isótopo pesado/isótopo ligero en una muestra con respecto a un estándar. La abundancia de isótopos estables relativa al estándar se expresa en partes por mil (‰) de acuerdo con la siguiente expresión:

$$\delta X = [(R \text{ muestra}/R \text{ estándar}) -1] \times 10^3$$

donde X es ^{13}C o ^{15}N y R corresponde a la fracción isótopo pesado/isótopo ligero ($^{13}C/^{12}C$ o $^{15}N/^{14}N$) en la muestra y en el estándar, respectivamente. Los estándares aceptados por la comunidad internacional incluyen Pee Dee Belemnite (PDB) para el ^{13}C y el nitrógeno atmosférico (aire) para el ^{15}N (Werner & Brand, 2001). Debido a que estos estándares internacionales son arbitrarios, algunas proporciones de isótopos son positivas (es decir, enriquecidas en el isótopo pesado con respecto al estándar) y otras son negativas (es decir, empobrecidas en el isótopo pesado con respecto al estándar).

El supuesto básico sobre el que se fundamenta la aplicación de los isótopos estables a los estudios de alimentación es que la señal isotópica corporal de los animales deriva directamente de aquella de los alimentos consumidos (proteínas, grasa y carbohidratos en el caso del $\delta^{13}C$ y principalmente proteínas en el caso del $\delta^{15}N$), por lo que la señal isotópica del organismo puede emplearse para conocer la importancia relativa de diferentes fuentes de alimento (Peterson & Fry, 1987; DeNiro & Epstein, 1978, 1981; Michener & Schell, 1994). Esta metodología permite integrar la señal isotópica de la totalidad de las presas asimiladas, evitando los sesgos de la digestibilidad de la presa inherentes al análisis de contenidos estomacales y de excrementos. Además, al requerir únicamente una pequeña muestra de tejido, se minimizan las molestias a los animales muestreados, por lo que el método es ampliamente usado en el caso de especies amenazadas (Michener & Schell, 1994; Crawford *et al*., 2008).

En un estudio trófico basado en el análisis de isótopos estables es muy importante la selección del tejido apropiado, ya que la composición isotópica de cada tejido cambia en el tiempo de acuerdo con su tasa de renovación, por lo que cada uno de ellos integra la señal isotópica de la dieta ingerida en un plazo de tiempo distinto (DeNiro & Epstein, 1978, 1981; Tieszen *et al*., 1983; Hobson, 1993; Hobson & Clark, 1992a, 1993; Hilderbrand *et al*., 1996; Kurle & Worthy 2002; Dalerum & Angerbjörn, 2005). Por ello, tejidos metabolicamente activos con una elevada tasa de renovación proporcionan información sobre los alimentos asimilados recientemente (ej. días en el caso de plasma y hígado), mientras que los tejidos con una tasa de renovación menor proporcionan información sobre la dieta de un período de tiempo mucho mayor (ej. semanas en el caso del músculo y sangre, meses o años en el caso de hueso) (Tieszen *et al*., 1983; Hobson & Clark, 1992a, 1993; Hilderbrand *et al*., 1996) algo especialmente útil para determinar los hábitos alimenticios a corto y a largo plazo. Los tejidos metabolicamente inactivos (ej. los tejidos de queratina como uñas, pelo, plumas, barbas, vibrisas) proporcionarán información sobre los alimentos asimilados en el momento de su formación, porque una vez que se vuelven inertes sus señales isotópicas permanecen inalteradas. Por tanto, en estos tejidos, a diferencia de los tejidos metabólicamente activos, el momento del muestreo es independiente de la información isotópica integrada en ellos (Schell *et al.*, 1989; Rubenstein & Hobson, 2004; Cherel *et al.*, 2009).

Una transferencia conservativa de la composición isotópica de la dieta al consumidor hace que la composición isotópica de los tejidos del animal refleje la de sus fuentes de alimento en una manera previsible, permitiendo así que los isótopos estables sean utilizados como trazadores en las redes tróficas (Hobson *et al*., 1994; Post, 2002).

Sin embargo existen cambios entre la señal isotópica de la dieta y los tejidos de los consumidores, como consecuencia de dos procesos principales: la asimilación bioquímica selectiva de los componentes dietarios con diferentes señales isotópicas y la discriminación isotópica (Hobson & Clark 1992b). Estos fenómenos producen un enriquecimiento o empobrecimiento de los isótopos pesados en relación con el isótopo ligero. El enriquecimiento ocurre cuando el isótopo estable más pesado se acumula en el tejido del consumidor en relación con la dieta, mientras que el isótopo más ligero es preferiblemente eliminado (ej. pérdida preferencial del $^{12}CO_2$ durante la respiración o del ^{14}N en forma de urea y absorción de compuestos de ^{13}C y ^{15}N durante la digestión) (Tieszen *et al*., 1983; Minagawa & Wada, 1984; DeNiro & Epstein, 1978, 1981; Michener & Schell, 1994). Por otro lado, el empobrecimiento se produce cuando se favorece el isótopo más ligero. Estos procesos de enriquecimiento o empobrecimiento se conocen en general como fraccionamiento o discriminación.

Por ello, para relacionar las concentraciones de isótopos en un tejido del consumidor con las de su dieta, es necesario establecer los valores de fraccionamiento isotópico para cada tipo de tejido. Esto se hace mediante experimentos controlados en cautividad bajo dieta constante.

Con esta información es posible predecir los valores dietéticos isotópicos de acuerdo a la ecuación:

$$D_t = D_d + \Delta_{dt}$$

donde D_t es la señal isotópica del tejido del consumidor, D_d es la señal isotópica de la dieta y Δ_{dt} es el factor de fraccionamiento entre dieta y tejido del consumidor (Hobson & Clark, 1992b; Post, 2002). Además, la estimación de los factores de fraccionamiento isotópico y de las diferencias en la señal isotópica entre los diferentes tejidos del consumidor permitirá su comparación (Hobson *et al*., 1996; Kurle, 2002; Jenkins *et al*., 2001; Dalerum & Angerbjörn, 2005).

Ahora bien, determinar la composición de la dieta no es la única utilidad de los isótopos estables en ecología. En toda cadena trófica, al pasar de un nivel trófico al siguiente, la materia orgánica asimilada se ve enriquecida en los isótopos pesados, tanto de carbono como de nitrógeno. La relación ^{15}N /^{14}N (expresado como $\delta^{15}N$) se enriquece ≈2-4‰, en función del tejido y de la especie, al pasar de un nivel trófico al siguiente, debido a la excreción preferencial del isótopo más ligero (Caut *et al.*, 2009). En el caso del carbono, la relación $^{13}C/^{12}C$ (expresado como $\delta^{13}C$) también tiende a aumentar con el nivel trófico, pero en menor medida que el $\delta^{15}N$: ≈0,8‰ en las redes tróficas de aguas costeras y ≈1,1‰ en las redes tróficas de mar abierto (France & Peters, 1997). El mayor enriquecimiento del $\delta^{15}N$ en comparación con el $\delta^{13}C$ hace del primero un buen indicador para determinar el nivel trófico de una especie comparando el $\delta^{15}N$ de sus tejidos con el de organismos de nivel trófico conocido del mismo ecosistema (Michener & Schell, 1994).

Por otra parte, las diferencias entre el $\delta^{13}C$ de los productores primarios hace de esto un buen indicador del hábitat. Las diferencias entre el $\delta^{13}C$ de los productores primarios se deben a la lenta difusión del CO_2 en el medio acuático y a la preferencia de las plantas por las moléculas de CO_2 portadoras del isótopo ligero (^{12}C), lo que las lleva a emplear moléculas con ^{13}C sólo cuando el isótopo ligero se ha agotado. Las plantas con una capa frontera más gruesa agotan más rápidamente el ^{12}C disponible, por lo que se ven obligadas a utilizar una mayor proporción de ^{13}C (France, 1995a). Como consecuencia, presentan valores de $\delta^{13}C$ superiores a los de las plantas con capa frontera más fina (France, 1995a). Al tener las células del fitoplancton una capa frontera más fina que los macrófitos, las redes tróficas bentónicas costeras se caracterizan por valores de $\delta^{13}C$ superiores a los de las redes tróficas pelágicas (France, 1995b). Por lo tanto este parámetro, al diferir entre varios tipos de productores primarios, proporciona información sobre la fuente de carbono que sustenta la red trófica a la que pertenece la especie de interés (Fry & Sherr, 1984; Michener & Schell, 1994; France, 1995a), algo especialmente útil para determinar si la especie tiene costumbres alimenticias costeras o pelágicas.

Por otro lado, la tasa de crecimiento de la planta también afecta al $\delta^{13}C$ (Michener & Schell, 1994), pues cuando ésta es elevada, los requerimientos de CO_2 de la planta aumentan y el CO_2 ligero presente en la capa frontera que rodea la planta se agota

rápidamente. Como consecuencia, la planta utiliza también el CO_2 pesado. En cambio, si la tasa de crecimiento es baja, la difusión desde el medio externo hacia la capa frontera garantiza el suministro constante de CO_2 ligero, por lo que la planta apenas utiliza CO_2 pesado y su $\delta^{13}C$ permanece en valores muy bajos. Esto hace que exista un amplio rango de valores de $\delta^{13}C$ entre los macrófitos de un mismo lugar, aunque el solapamiento con los valores de $\delta^{13}C$ del fitoplancton es escaso (France, 1995a). En cualquier caso, es necesario determinar los valores de $\delta^{13}C$ de los productores primarios de la zona para validar que estas generalizaciones se cumplen.

Aunque la utilidad del análisis de isótopos estables ha sido ampliamente documentada, algunas cuestiones limitan su uso, especialmente en el estudio del ambiente marino, donde la complejidad de los procesos bioquímicos y oceanográficos hace que la dinámica isotópica de las redes tróficas marinas sea más difícil de descifrar que en los ecosistemas terrestres (Michener & Schell, 1994). Además, se conoce poco sobre la variabilidad geográfica de la señal isotópica. Esta falta de conocimiento limita la aplicabilidad del análisis de isótopos estables para entender las relaciones tróficas de especies con grandes áreas de distribución, así como para descifrar sus movimientos migratorios. Por lo tanto, ampliar nuestro conocimiento de los paisajes isotópicos marinos y los factores que influyen en las señales isotópicas de los organismos marinos constituyen una forma para optimizar la eficacia de esta metodología en el estudio de los ambientes marinos (West *et al*., 2006).

OBJETIVOS

La presente tesis doctoral tiene como objetivo general determinar hasta que punto la recuperación del león marino del sur se ha visto afectada por el desarrollo de la pesca industrial.

Para poder hacerlo ha sido necesario determinar en primer lugar como se ve afectada la dieta por la edad, el sexo y el estado reproductivo, aspectos muy poco documentados en la especie. La hipótesis inicial era que la masa corporal condiciona la capacidad de buceo y por lo tanto el uso relativo de recursos pelágicos y bentónicos. Ahora bien, tras el parto, la necesidad de estar cerca de las colonias podría obligar a las hembras a utilizar más presas bentónicas de lo habitual. En el primer capítulo se han evaluado ambas hipótesis mediante el uso de isótopos estables. Para ello (Sección 1) se analizaron los isótopos estables de carbono y nitrógeno en los huesos del cráneo de individuos de ambos sexos y diferentes edades para comprobar si: 1) el consumo de presas bentónicas se incrementa desde el destete hasta la edad adulta dentro de cada sexo; 2) las hembras adultas tienen una dieta más pelágica que los machos adultos; y 3) el consumo de presas bentónicas también aumenta con la edad en adultos que han terminado el crecimiento. A continuación (Sección 2), se determinaron las concentraciones de isótopos estables de carbono y nitrógeno en el suero y las células sanguíneas de crías lactantes de león marino del sur, para reconstruir la dieta de la madre durante la parte final de la gestación y el inicio de la lactancia, y así comprobar si las hembras adoptan una dieta más bentonita y costera tras el parto con el fin de reducir la duración de los viajes de alimentación y reducir así el tiempo durante el cual las crías permanecen desatendidas en la playa.

Una segunda cuestión a resolver era cómo se veían afectados el crecimiento y la supervivencia de las crías por la calidad de la dieta materna y por las condiciones de la colonia de cría. La hipótesis de partida fue que el éxito reproductivo de las hembras dependía por un lado de la dieta y por otro del tamaño de la colonia. En primer lugar, la mayor calidad nutricional de las presas pelágicas en comparación con las presas bentónicas haría que las hembras con una dieta más pelágica produjeran leche más rica energéticamente y por lo tanto sus crías crecieran más rápido. Por otra parte, la supervivencia de las crías podía depender del tamaño de la colonia, ya que la

vulnerabilidad al infanticidio por parte de machos subadultos podría disminuir en las colonias de mayor tamaño. En el segundo capítulo se han evaluado ambas cuestiones. Para ello (Sección 3), se utilizaron concentraciones de isótopos estables de carbono y nitrógeno en el suero y las células sanguíneas de crías lactantes como indicadores de la composición de la dieta de la madre, relacionándolas con la tasa de crecimiento de las crías para así comprobar la influencia de la estrategias de alimentación de las hembras lactantes del león marino del sur sobre el crecimiento de las crías. Por otro lado (Sección 4), se analizó el efecto del tamaño de la colonia sobre la calidad de la dieta de las hembras lactantes, la tasa de crecimiento, la respuesta inmunitaria y la tasa de mortalidad de las crías

Una vez aclaradas las cuestiones anteriores se estaba en condiciones de evaluar cómo había cambiado la dieta de los leones marinos al desarrollarse la pesca industrial y si los posibles cambios estaban relacionados con una reducción de la capacidad de carga del medio, cuestiones que se han afrontado en el tercer capítulo. Por una parte (Sección 5), se analizaron los isótopos estables de carbono y nitrógeno en los huesos del cráneo de individuos de ambos sexos y similar edad a lo largo del siglo XX para determinar cómo había cambiado la dieta en dicho período. Por otro lado (Sección 6), se utilizaron datos craneométricos de individuos adultos de ambos sexos correspondientes a períodos con contrastante disponibilidad de alimento a lo largo del siglo XX, para evaluar cómo la recuperación de la población tras el cese de su explotación comercial y el desarrollo de la industria pesquera han afectado al crecimiento somático y si existe una correlación inversa entre el crecimiento somático y la densidad de la población.

Capítulo 1. Incidencia de la edad, el sexo y el estado reproductivo sobre la dieta del león marino del sur

Sección 1. *Cambios ontogénicos en la dieta de los leones marinos del sur*

- M. Drago, L. Cardona, E.A. Crespo, A. Aguilar (2009) Ontogenic dietary changes in South American sea lions. *Journal of Zoology*, 279: 251-261.

Sección 2. *Cambio en la estrategia de alimentación de las hembras del león marino del sur (Carnivora, Pinnipedia) tras el parto*

- M. Drago, L. Cardona, E.A. Crespo, N. García, S. Ameghino, A. Aguilar (2010) Change in the foraging strategy of female South American sea lions (Carnivora, Pinnipedia) after parturition. *Scientia Marina*, in press.

Capítulo 2. Incidencia de la dieta materna y dimensión de las colonias sobre el crecimiento y la supervivencia de las crías

Sección 3. *Influencia de la dieta materna, revelada mediante isótopos estables, sobre el crecimiento de las crías del león marino del sur*

- M. Drago, L. Cardona, A. Aguilar, E.A. Crespo, S. Ameghino, N. García (2009) Diet of lactating South American sea lions, as inferred from stable isotopes, influences pup growth. *Marine Mammal Science*, in press (DOI: 10.1111/j.1748-7692.2009.00321.x).

Sección 4. *Influencia del tamaño de las colonias sobre el bienestar y la supervivencia de las crías en los leones marinos del sur*

- M. Drago, L. Cardona, N. García, S. Ameghino, A. Aguilar (2010) Influence of colony size on pup fitness and survival in South American sea lions. *Marine Mammal Science*, in press.

Capítulo 3. Efecto de los cambios demográficos a largo plazo sobre la dieta y el crecimiento somático

Sección 5. *Cambio histórico de la dieta del león marino del sur en la Patagonia revelado por análisis isotópico*

- M. Drago, E.A. Crespo, A. Aguilar, L. Cardona, N. García, S.L. Dans, N. Goodall (2009) Historic diet change of the South American sea lion in Patagonia as revealed by isotopic analysis. *Marine Ecology Progress Series*, 384: 273-286.

Sección 6. *La reducción del tamaño del cráneo en los leones marinos del sur revela un crecimiento densodependiente durante la recuperación de la población*

- M. Drago, L. Cardona, E.A. Crespo, M.F. Grandi, A. Aguilar (2010) Reduction of skull size in South American sea lions reveals density-dependent growth during population recovery. *Marine Ecology Progress Series*, submitted.

Informe del director

El doctorando **Massimiliano Drago** presenta en su tesis doctoral titulada **"Dieta y dinámica poblacional del león marino del sur (*Otaria flavescens*) en Patagonia"**, seis artículos de gran calidad científica, presentados y/o publicados todos ellos en revistas científicas internacionales de gran prestigio incluidas en el *Science Citation Index* (SCI).

A continuación se detalla la contribución que ha realizado el doctorando en cada uno de los artículos, así como el factor de impacto de cada uno de ellos, tal y como están publicados por el *Institute for Scientific Information* (ISI) y listados en el *Journal Citation Reports* (JCR) correspondiente a 2008:

• **Ontogenic dietary changes in South American sea lions**
M. Drago, L. Cardona, E.A. Crespo, A. Aguilar (2009)
Journal of Zoology, 279: 251-261
Factor de impacto (2008): 1,669
Diseño del trabajo: **M.D.**, L.C., E.A.C., A.A.
Muestreo: **M.D.**, E.A.C.
Análisis de las muestras: **M.D.**, L.C.
Redacción científica: **M.D.**, L.C.

• **Change in the foraging strategy of female South American sea lions (Carnivora, Pinnipedia) after parturition**
M. Drago, L. Cardona, E.A. Crespo, N. García, S. Ameghino, A. Aguilar (2010)
Scientia Marina, en prensa
Factor de impacto (2008): 1,075
Diseño del trabajo: **M.D.**, L.C., E.A.C., A.A.
Muestreo: **M.D.**, E.A.C., N.G., S.A.
Análisis de las muestras: **M.D.**, L.C.
Redacción científica: **M.D.**, L.C.

• **Diet of lactating South American sea lions, as inferred from stable isotopes, influences pup growth**

M. Drago, L. Cardona, A. Aguilar, E.A. Crespo, S. Ameghino, N. García (2009)

Marine Mammal Science, en prensa (DOI: 10.1111/j.1748-7692.2009.00321.x)

Factor de impacto (2008): 1,787

Diseño del trabajo: **M.D.**, L.C., A.A., E.A.C.

Muestreo: **M.D.**, E.A.C., S.A., N.G.

Análisis de las muestras: **M.D.**, L.C.

Redacción científica: **M.D.**, L.C., A.A.

• **Influence of colony size on pup fitness and survival in South American sea lions**

M. Drago, L. Cardona, N. García, S. Ameghino, A. Aguilar (2010)

Marine Mammal Science, en prensa

Factor de impacto (2008): 1,787

Diseño del trabajo: **M.D.**, L.C., E.A.C., A.A.

Muestreo: **M.D.**, E.A.C., N.G., S.A.

Análisis de las muestras: **M.D.**, L.C.

Redacción científica: **M.D.**, L.C.

• **Historic diet change of the South American sea lion in Patagonia as revealed by isotopic analysis**

M. Drago, E.A. Crespo, A. Aguilar, L. Cardona, N. García, S.L. Dans, N. Goodall (2009)

Marine Ecology Progress Series, 384: 273–286

Factor de impacto (2008): 2,631

Diseño del trabajo: **M.D.**, E.A.C., A.A., L.C.

Muestreo: **M.D.**, E.A.C., N.G., S.L.D., N.G.

Análisis de las muestras: **M.D.**, L.C.

Redacción científica: **M.D.**, A.A., L.C.

• **Reduction of skull size in South American sea lions reveals density-dependent growth during population recovery**

M. Drago, L. Cardona, E.A. Crespo, M.F. Grandi, A. Aguilar (2010)

Marine Ecology Progress Series, enviado

Factor de impacto (2008): 2,631

Diseño del trabajo: **M.D.**, L.C., E.A.C., A.A.

Muestreo: **M.D.**, E.A.C., M.F.G.

Análisis de las muestras: **M.D.**, L.C.

Redacción científica: **M.D.**, L.C.

De la misma manera se informa de que ninguno de los coautores participantes en los artículos que componen esta tesis ha utilizado, implícitamente o explícitamente, estos trabajos para la elaboración de su propia tesis doctoral.

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Discusión General y Conclusiones

Influencia del sexo y la edad sobre la dieta

El tamaño corporal de los vertebrados marinos que respiran aire es un parámetro que condiciona la capacidad de buceo, ya que un mayor tamaño corporal permite un mayor almacenamiento de oxígeno y por lo tanto un mayor tiempo de buceo (Kooyman, 2009). Por este motivo, las especies de pinnípedos con mayor masa corporal realizan generalmente buceos más largos y/o profundos que las de menor masa (Gentry *et al*., 1986; Kooyman, 1989; Costa, 1991, 1993; Costa *et al*., 2004). Así mismo, los machos de las especies de pinnípedos altamente dimórficas muestran una mayor capacidad de buceo que las hembras (Le Boeuf *et al*., 1996, 2000; Meynier *et al*., 2008). En este contexto, cabría esperar un uso más intenso de los recursos bentónicos por parte de los machos de león marino del sur que por parte de las hembras. Así mismo, se esperaría un incremento en el uso de los recursos tróficos bentónicos con el aumento de la talla y la edad dentro de un mismo sexo. Esta mejora podría continuar con la edad más allá de la finalización del crecimiento corporal si se produjera una mejoría en el control de la demanda de oxigeno con la edad (Rea & Costa, 1992; Ponganis *et al*., 1993; Greaves *et al*., 2005; Fowler *et al*., 2007a,b).

Los datos isotópicos aquí presentados no permiten una reconstrucción precisa de la composición de la dieta de los leones marinos del sur, porque se desconoce el fraccionamiento entre la señal isotópica de las presas potenciales y el tejido óseo del león marino del sur. Existen datos experimentales para otras especies de pinnípedos, pero se refieren a otros tejidos (Hobson *et al*., 1996; Kurle, 2002). Estos datos tampoco permiten determinar el tiempo o la profundidad de buceo a la cual se alimentan los leones marinos. Sin embargo, el paisaje isotópico de los ecosistemas marinos de la provincia de Chubut indica un incremento de los valores del $\delta^{13}C$ al pasar de especies pelágicas a especies bentónicas y un incremento de los valores del $\delta^{15}N$ con el nivel trófico. En estas condiciones, un incremento del valor de $\delta^{13}C$ del material óseo de los leones marinos a lo largo de la ontogenia reflejaría un incremento en la proporción de presas bentónicas consumida y por lo tanto reflejaría una mejora de la capacidad de buceo.

Los resultados obtenidos apuntan en esta línea, pues son coherentes con un aumento del consumo de presas bentónicas entre el destete y la pubertad en ambos sexos. Este patrón se mantiene al llegar a la edad adulta, excepto en los machos seniles, lo que apoya la hipótesis de que la alimentación está estrechamente vinculada a la mejora de las habilidades de buceo como resultado de un aumento del tamaño corporal (Le Boeuf *et al*., 1996; Horning & Trillmich, 1997). Las diferencias en el tamaño corporal explican también porqué los machos tienen una dieta mucho más bentónica que las hembras del mismo estadio de desarrollo, salvo en los ejemplares seniles. Esta interpretación de los resultados coincide con la de estudios previos basados en el uso de TDR (registrador tiempo-profundidad) y telemetría satelital, según los cuales los leones marinos del sur de ambos sexos son capaces de comer tanto cerca del fondo marino como en la columna de agua (Werner & Campagna, 1995; Campagna *et al*., 2001), utilizando los machos hábitat más profundos que las hembras (Campagna *et al*., 2001).

Hay que tener en cuenta, no obstante, que esta interpretación de los cambios ontogénicos en la proporción de isótopos estables de los huesos depende en gran medida de la tasa de renovación real del tejido óseo, que determina el marco temporal durante el cual se integra la señal isotópica de la dieta (Tieszen *et al*., 1983; Hobson & Clark, 1992a; Hobson, 1993). Por otra parte, las crías lactantes de pinnípedos están más enriquecidas en ^{15}N que sus madres, ya que se produce un fraccionamiento entre los tejidos corporales de las hembras lactantes y los de sus crías, mientras que el patrón es menos claro para el $\delta^{13}C$ y pueden ser especie-dependiente (Newsome *et al*., 2006; Ducatez *et al*., 2008). La evidencia experimental disponible para el león marino del sur indica que las crías lactantes de esta especie están, efectivamente, enriquecidas en ^{15}N con respecto a sus madres, sin que exista fraccionamiento significativo entre la madre y la cría para el carbono (Drago M., datos no publicados). Resultados similares se han observado experimentalmente en otros mamíferos (Jenkins *et al*., 2001).

En este escenario, los tejidos de la cría formados durante la lactancia están más enriquecidos no sólo con respecto a los de su madre, sino también con respecto a los que sintetice tras el destete, aunque la señal de lactancia ira desapareciendo con el tiempo. Al menos en otra especie de otárido (ej. *Callorhinus ursinus*), la señal de la lactancia decae después del destete y desaparece completamente del tejido óseo al cabo de 8-10 meses tras

el destete (Newsome *et al*., 2006), lo que da idea de la tasa de renovación del tejido óseo en esta especie y por lo tanto del marco de integración temporal de la señal isotópica del hueso. No obstante, es posible que la tasa de renovación ósea disminuya con la edad y por lo tanto las proporciones de isótopos estables en el hueso se integren en un marco temporal de mayor duración de los leones marinos de mayor edad, especialmente una vez finalizado el crecimiento somático.

La persistencia de una señal de lactancia podría explicar los valores de $\delta^{15}N$ excepcionalmente elevados observados en algunos de los individuos inmaduros analizados en la presente tesis doctoral, especialmente hembras, ya que los leones marinos del sur son destetados a los 6-10 meses de edad, siendo los machos destetados antes que las hembras (Crespo, 1988). De acuerdo con esto y con el tiempo necesario para eliminar la marca de lactancia en otras especies de otáridos arriba indicado, los leones marinos del sur mayores de 20 meses (1,8 años) deberían estar completamente libres de la señal de lactancia, mientras que las proporciones de isótopos estables de los individuos más jóvenes serían una mezcla de la señal de lactancia y de su propia dieta. Sólo tres de los 10 machos inmaduros incluidos en el presente estudio eran menores de 2 años y, por tanto, susceptibles de exhibir restos de la señal de la lactancia, mientras que hasta seis de las hembras inmaduras podrían haber exhibido restos de la señal de la lactancia, al ser menores de 2 años. La persistencia de algunos restos de la señal de lactancia en algunos de los inmaduros analizados probablemente explica por qué la desviación estándar del $\delta^{15}N$ de los leones marinos inmaduros de cada sexo es mayor que la de los restantes estadios de desarrollo y por qué el $\delta^{15}N$ de las hembras inmaduras era mayor que el de las hembras en los otros estadios de desarrollo. Sin embargo, la interpretación de los cambios ontogénicos en las habilidades de buceo de los leones marinos del sur se deriva de los datos $\delta^{13}C$, que no estarían afectados por la persistencia de la señal de lactancia al no existir fraccionamiento madre-cría.

Una vez aclarado el posible efecto de la señal de lactancia sobre la señal isotópica, es necesario remarcar que el crecimiento del tamaño corporal resulta insuficiente para explicar el contínuo incremento del consumo de presas bentónicas tras alcanzarse la madurez sexual, pues los leones marinos del sur tienen un crecimiento en longitud determinado (Rosas *et al*., 1993; presente estudio) y por lo tanto no cabe esperar un

incremento de masa relevante durante la vida adulta. Se desconoce la relación entre longitud y peso en el león marino del sur, aunque se sabe que en algunos otáridos el peso se estabiliza tras la madurez sexual a edades más tardías que la longitud corporal (Winship *et al*., 2001), mientras en las hembras adultas de otras especies aumenta ligeramente con la edad (Chilvers *et al*., 2006). Sin embargo, es poco probable que un patrón similar en la relación entre edad y peso para los leones marinos del sur pueda explicar el incremento continuado del consumo de presas bentónicas a lo largo de la vida adulta de esta especie, dada la alta variabilidad en el peso corporal de los leones marinos adultos de otras especies (Winship *et al*., 2001; Chilvers *et al*. , 2006), la ausencia de una correlación entre la edad y la profundidad de buceo en las hembras adultas de otras especies (Chilvers *et al*., 2006) y la amplia variabilidad en el peso esperada para las hembras de leones marinos del sur de acuerdo con la variabilidad de longitud (Rosas *et al*., 1993; presente estudio).

Un mayor acceso a las presas bentónicas con la edad después de la madurez sexual puede explicarse alternativamente por una mejora del control sobre los mecanismos fisiológicos implicados en el buceo. Los pinnípedos jóvenes tienen un acceso limitado a los recursos bentónicos no sólo porque tienen una menor reserva de oxígeno, sino también porque parecen incapaces de regular el ritmo cardíaco, la respiración, la vasoconstricción o la temperatura corporal tan eficazmente como hacen los adultos (Rea & Costa, 1992; Ponganis *et al*., 1993; Greaves *et al*., 2005; Fowler *et al*., 2007a,b). Como consecuencia, los pinnípedos jóvenes dependen con más frecuencia que los adultos de la energía producida anaeróbicamente después de un buceo largo y profundo (Kooyman *et al*., 1983; Burns, 1999). Esto es cierto incluso en el caso de especies en las que los adultos se alimentan en el fondo del mar y a menudo exceden sus límites de buceo aeróbico (Costa & Gales, 2000, 2003; Costa *et al*., 2001; Chilvers *et al*., 2006). En consecuencia, se cree que el aumento del consumo de presas bentónicas durante la vida adulta del león marino del sur puede depender del aprendizaje.

La reducción del consumo de presas bentónicas en los machos seniles es, sin embargo intrigante, y sugiere una disminución del rendimiento de buceo, tal vez debido a un debilitamiento fisiológico causado por una inversión muy alta de energía en la reproducción (Campagna & Le Boeuf, 1988). Esta hipótesis está respaldada por un pico de mortalidad de los machos de león marino del sur a los 11 años de edad (Crespo, 1988) y

por una posible reducción en el peso corporal en los animales seniles, tal como ha sido reportado para otras especies (Winship *et al*., 2001). Sin embargo, sería necesario conocer con detalle la relación peso-edad en esta especie para confirmar esta hipótesis.

Influencia del estado reproductivo sobre la dieta de las hembras

Tal como se ha explicado anteriormente, la comparación de la señal isotópica del tejido óseo de machos y hembras ha revelado que los primeros presentan una alimentación más bentónica de las segundas. Sin embargo un estudio basado el análisis de contenidos estomacales de individuos varados en el norte de la Patagonia, sugiere el patrón opuesto (Koen Alonso *et al*., 2000). Esta contradicción podría explicarse si el estado reproductivo condicionara la estrategia de alimentación de las hembras, comportándose estas como depredadores epipelágicos durante la mayor parte del año y adoptando una dieta más costera y bentónica tras el parto, ya que la mayor parte de las hembras analizadas por Koen Alonso *et al*. (2000) habían varado durante la temporada de cría. Este cambio de estrategia permitiría a las hembras lactantes encontrar un punto de equilibrio entre la necesidad de amamantar a las crías y la de ir al mar a alimentarse.

Las hembras lactantes de león marino del sur, al igual que las de otros otáridos, alternan viaje de alimentación con estancias en tierra durante las cuales amamantan a las crías (Campagna & Le Boeuf, 1988). Durante los viajes de alimentación, las crías permanecen sin atención mientras sus madres están en el mar y pueden morir de inanición (Soto *et al*., 2004; Reid & Forcada, 2005) o por el comportamiento agonístico de los adultos y subadultos de la colonia (Campagna *et al*., 1988b, 1992; Higgins & Tedman, 1990). En este escenario, cabe esperar que las hembras lactantes modifiquen su comportamiento de alimentación (ej. elección de la presa, tiempo de alimentación) con el fin de reducir lo más posible la duración del viaje y al mismo tiempo intentar satisfacer sus propias necesidades de alimentación (Costa, 2008). Estos compromiso pueden limitar las hembras a alimentarse más cerca de la colonia durante la fase inicial de la lactancia, provocando así un cambio en la dieta después del parto que las puede llevar a explotar presas no consumida durante el resto del año (Trillmich, 1990; Merrick & Loughlin, 1997; Boyd *et al*., 1998, 2002; Melin *et al*., 2000).

Los datos derivados del análisis de la señal isotópica del suero y de las células sanguíneas de crías lactantes corroboran esta hipótesis, pues la reconstrucción de la dieta revela una fuerte dependencia de las especies pelágicas durante las fases finales de la gestación y su substitución parcial por especies bentónicas tras el parto. Ahora bien, la contribución particular de cada una de las especies pelágicas a la dieta resulta difícil de determinar, debido a las similitudes existentes entre las señales isotópicas de la merluza argentina (*Merluccius hubbsi*) con una talla inferior a los 30 cm de longitud y la de anchoita (*Engraulis anchoita*), así como entre la señal isotópica de la merluza argentina de más de 30 cm de longitud y la de calamarete (*Loligo sanpaulensis*). Como consecuencia, el programa utilizado para calcular la contribución de cada especie a la dieta atribuye aproximadamente la misma relevancia a los miembros de cada uno de los dos grupos anteriores, aunque la anchoita y el calamarete son mucho más escasos en los contenidos estomacales que la merluza argentina de cualquier tamaño (Koen Alonso *et al*., 2000). El resultado de la reconstrucción dietaria también revela una aportación mucho mayor de pulpito tehuelche (*Octopus tehuelchus*) a la dieta de las hembras de leones marinos después del parto que el análisis del contenido estomacal (Koen Alonso *et al*., 2000). Esto es debido quizás al pequeño tamaño del pulpito tehuelche en comparación con el pulpo colorado (*Enteroctopus megalocyathus*) y por lo tanto a la dificultad de identificar los diminutos picos en el contenido del estómago.

Pero al margen de estas discrepancias sobre la contribución relativa de cada especie particular, las diferencias entre las señales isotópica de suero y células sanguíneas, una vez corregidas de conformidad con el enriquecimiento isotópico previsto, revelan claramente un cambio notable después del parto hacia presas bentónicas costera a expensas de las presas pelágicas, similar al documentado para otras especies de pinnípedos (Chilvers *et al*., 2005). De este modo, las hembras reducirían la duración de los viajes de alimentación y podrían pasar más tiempo con las crías.

Esta hipótesis se ve apoyada por los resultados de un estudio de telemetría según el cual la duración de los viajes de alimentación de las hembras lactantes en el norte de la Patagonia están significativamente correlacionados con la distancia recorrida, aunque las hembras que se alimentan en alta mar y que viajaban más lejos nadan más rápidamente de aquellas que se alimentaban cerca de la costa (Campagna *et al*., 2001). Además, en Perú se

ha documentado una correlación positiva entre la duración de los viajes de alimentación de hembras lactantes de león marino del sur y la tasa de mortalidad de las crías (Soto *et al*., 2004, 2006).

En este escenario, la estrategia de alimentación de las hembras de león marino tras el parto no busca maximizar la ingesta energética, sino minimizar el tiempo que la hembra pasa en el mar. No obstante, esta estrategia de lactancia podría comprometer la tasa de crecimiento de las crías si la calidad nutricional de las presas bentónicas fuera inferior a la de las presas pelágicas.

Influencia de la dieta materna sobre la tasa de crecimiento de las crías

Tras el parto, las hembras lactantes necesitan satisfacer el alto requerimiento de energía exigido por la lactancia, especialmente durante las primeras semanas, cuando necesitan proporcionar a sus crías leche rica en lípidos. Los fócidos no se alimentan durante la lactancia, por lo que las hembras utilizan sus reservas lipídicas para sintetizar una leche extraordinariamente grasa (Riedman, 1990). No sucede lo mismo con las hembras de otáridos, que deben alimentarse durante la lactancia y por lo tanto son sensibles a la calidad del alimento durante ese período (Riedman, 1990; Costa *et al*., 2006). En este contexto, parecería razonable que las hembras de otáridos incrementen el consumo de presas de elevado valor nutritivo tras el parto.

El análisis del contenido energético de las presas potenciales presentes en el norte de la Patagonia revela que las presas pelágicas por lo general tienen una mayor densidad de energía que las presa bentónicas y un contenido proteico similar (Eder & Lewis, 2005). Los resultados obtenidos durante la realización de la presente tesis doctoral confirman dicha hipótesis, auque la densidad de energía del lenguado (*Paralichthys isosceles*) es similar a la de la mayoría de las presas pelágicas potenciales y superior a la de otras especie bentónicas como el pulpo colorado (*Enteroctopus megalocyathus*), el pulpito tehuelche (*Octopus tehuelchus*) y la raneya (*Raneya brasiliensis*). Por lo tanto, cabría esperar un aumento del consumo de presas pelágicas tras el parto si las hembras optaran por maximizar la energía. Sin embargo, los resultados del presente estudio revelan un aumento en el consumo de las especies bentónicas tras el parto, tal como se ha indicado

anteriormente. Ciertamente, algunas especies de pinnípedos optan por consumir presas de gran tamaño para compensar una baja densidad energética (Costa & Gales, 2003; Chilvers *et al*., 2006), pero los datos disponibles indican que esto no sucede en el caso de las hembras lactantes de león marino del sur. Los resultados del análisis de isótopos estables indican que después del parto las hembras lactantes de león marino del sur consumen una gran cantidad de pequeñas especies bentónicas, como el pulpito tehuelche y la raneya, en lugar de especies más grandes como el pulpo colorado y el lenguado. Lo mismo sucede en las Islas Malvinas (Falkland Islands), donde el análisis de excrementos indica que las hembras lactantes de león marino del sur consumen principalmente pequeñas especies de peces nototénidos bentónicos (Thompson *et al*., 1998). Esta intensa utilización de presas de pequeño tamaño y baja densidad energética podría explicar el descenso del contenido de grasa de la leche en las hembras de león marino del sur durante el transcurso de la lactancia (Werner *et al*., 1996), cuando en los fócidos sucede lo contrario (Riedman, 1990).

Los datos obtenidos durante la realización de la presente tesis doctoral indican que el crecimiento de las crías se ve fuertemente influido por la calidad de la dieta de las hembras, pues las crías de hembras que se alimentaban principalmente de presas pelágicas, según indican los valores de $\delta^{13}C$ y $\delta^{15}N$, crecían más rápidamente que aquellas cuyas madres se alimentaban principalmente de presas bentónicas costeras. Una relación similar se ha observado en los pingüinos de Magallanes *Spheniscus magellanicus* de la Patagonia (Forero *et al*. 2002a,b). De todos modos, es necesario tener presente el efecto del ayuno sobre la señal isotópica para interpretar correctamente los resultados (Hobson *et al*., 1993; Cherel *et al*., 2005; pero ver Kempster *et al*., 2007), pues las crías lactantes de los leones marinos del sur ayunan de 1 a 10 días entre una toma y otra, mientras las madres se alimentan en el mar (Campagna & Le Boeuf 1988, Campagna *et al*., 2001).

Los datos publicados hasta la fecha sobre los efectos del ayuno en la señal isotópica de la sangre de endotermos indican que tras 25 días de ayuno se produce tan sólo un ligero aumento del $\delta^{15}N$ en el plasma (0,70‰) y un incremento aun menor del $\delta^{15}N$ de las células sanguíneas (0,24‰), mientras que no se observa ningún efecto sobre el $\delta^{13}C$ de las células sanguíneas y sólo un ligero descenso (-0,71‰) en el $\delta^{13}C$ de plasma (Cherel *et al*., 2005). En este escenario, es poco probable que una diferencia de pocos días en la duración del ayuno de las crías pueda explicar la notable variabilidad observada en el $\delta^{15}N$ del suero de

las crías (de 21,5‰ a 24,0‰). Por otro lado, si la variabilidad del $\delta^{13}C$ del suero de las crías estudiadas hubiera sido causada por las diferencias en la duración del período de ayuno entre periodos de lactancia, el $\delta^{13}C$ del suero de las crías debería disminuir a medida que aumentaba la duración del período de ayuno y la tasa de crecimiento disminuía. Sin embargo, los resultados indican que el $\delta^{13}C$ del suero de las crías aumentó al mismo tiempo que la tasa de crecimiento de las crías disminuía.

Por otra parte, la señal isotópica de las células sanguíneas, principalmente sintetizadas uno o dos meses antes del parto (Hobson & Clark, 1993; Hilderbrand *et al*., 1996) y por lo tanto independiente de la duración de los episodios de ayuno entre la lactancia, reveló un grado de variabilidad similar a aquella del suero. Esta similitud confirma que la variabilidad observada en la señal isotópica de suero no está relacionada con la variabilidad en la duración de los viajes de alimentación de las hembras y por lo tanto con el ayuno. En realidad, la correlación existente entre la señal isotópica de suero y la de las células sanguíneas indica que las hembras difieren en su dieta ya durante la fase final de la lactancia y que la posición relativa de cada hembra en un gradiente pelágico-bentónico se mantiene sin cambios después del parto. La razón de esta variabilidad intrapoblacional probablemente refleja diferencias en el comportamiento de alimentación individual, que depende de la edad y/o del tamaño corporal. Como se ha explicado anteriormente, los pinnípedos con mayor masa corporal presentan mejores aptitudes de buceo y pueden explotar intensamente los recursos bentónicos (Costa, 1991). Recientemente, Chilvers *et al*. (2006) y Villegas Amtmann *et al*. (2008) también han observado diferencias intraespecíficas en el comportamiento de buceo de hembras lactantes de león marino de Nueva Zelanda (*Phocarctos hookeri*) y de león marino de Galápagos (*Zalophus wollebaeki*) causada por las diferencias en la masa corporal. Además, las habilidades de buceo en pinnípedos continúan mejorando una vez finalizado el crecimiento somático y por lo tanto también aumentan con la edad la duración de las inmersiones, la profundidad de buceo, la distancia de los viajes y la velocidad de natación (Horning & Trillmich, 1997; Bekkby & Bjørge, 2000; Chilvers *et al*., 2005, 2006).

En cualquier caso, una dieta basada en presas pelágicas implica dejar a la cría sola en la playa durante un período de tiempo más largo, lo que podría acarrear una mayor tasa de mortalidad (Soto *et al*., 2004, 2006). Por lo tanto, las hembras se enfrentan al

compromiso entre escoger presas bentónicas costeras de menor calidad a cambio de pasar más tiempo con la cría, o bien utilizar presas pelágicas de mayor calidad y arriesgarse a perder la cría debido a las agresiones intraespecíficas.

A este respecto cabe remarcar que el infanticidio por parte de machos subadultos es la principal causa de mortalidad de crías de león marino del sur, al margen del ayuno excesivamente prolongado (Campagna *et al*., 1992). Los datos disponibles indican que la tasa de mortalidad de crías es mayor en unidades de cría aisladas, formada por un macho adulto y una hembra, que en colonias integradas por varias unidades de cría (Campagna *et al*., 1992). Por otra parte, Crespo *et al*. 2002 han indicado una menor tasa de supervivencia de las crías en colonias de reciente formación, cuya estructura social difiere de la de las colonias clásicas. En este contexto, es posible que la presión de las hembras para permanecer el mayor tiempo posible junto a la cría disminuya en contextos en los cuales la tasa de supervivencia aumente, es decir, en colonias de mayor tamaño y más antiguas.

Influencia del tamaño de la colonia en la tasa de supervivencia de las crías

La recuperación de la población del león marino del sur en el norte de la Patagonia ha sido un proceso a dos etapas (Crespo & Pedraza, 1991; Dans *et al*., 2004; Grandi *et al*., 2008). Inicialmente, las colonias que no habían sido extirpadas durante la explotación comercial crecían lentamente y no se fundaron nuevas colonias. La situación cambió a partir de 1990, cuando la tasa de producción de crías en las colonias originales se aceleró y se establecieron nuevas áreas de cría en sus proximidades. Estas nuevas colonias fueron inicialmente áreas de descanso utilizadas principalmente por ejemplares jóvenes, pero con los años su estructura social cambió y se aproximó a la de las colonias originales (Grandi *et al*., 2008). Aunque la tasa de supervivencia de las crías en estas nuevas colonias fue menor que en las originales, y por lo tanto su tasa de autoreclutamiento fue baja, las nuevas colonias crecieron más rápido que las colonias originales (Crespo *et al*., 2002) debido a la incorporación de hembras primerizas nacidas en estas últimas (Grandi *et al*., 2008).

Este proceso de expansión está en consonancia con la hipótesis de que la competencia intraespecífica por los recursos en las abarrotadas colonias originales promovería la dispersión de hembras primerizas hacia nuevas colonias en zonas aledañas

(Grandi *et al*., 2008). Cassini & Fernández-Juricic (2003) propusieron que el acoso intraespecífico era el factor más influyente en la selección de las colonias de parte de las hembras de león marino del sur. Por una parte, el acoso por parte de los machos hacia las hembras disminuye con el tamaño del grupo reproductor, al tiempo que aumenta la interaccione entre hembras. En teste contexto, Cassini & Fernández-Juricic (2003) hipotetizaron que el acoso intraespecífico se traduciría en una mayor mortalidad de las crías y que las hembras abandonarían las colonias para experimentar un menor acoso y así aumentar las posibilidades de supervivencia de las crías. Por lo tanto, la mortalidad de las crías debido a interacciones entre hembras sería un fenómeno denso-dependiente. En cambio, la mortalidad de las crías debido a los ataques de machos inmaduros sería un fenómeno relacionado negativamente con el tamaño de la colonia (Campagna *et al*., 1992).

Los resultados obtenidos durante la realización de la presente tesis doctoral indican que la tasa de supervivencia de las crías aumenta con el tamaño de la colonia, lo que sugiere que la mortalidad de las crías resultantes de los acosos entre los adultos es mucho menos importante que el infanticidio por parte de los machos subadultos. Además, los datos sobre la tasa de crecimiento de las crías durante las tres primeras semanas de vida indican una relación negativa entre el tamaño de la colonia y la tasa de crecimiento. Se sabe que la tasa de crecimiento de las crías está influida no sólo de la provisión de alimento por parte de las madres (Trillmich & Ono, 1991; Bester & Van Jaarsveld, 1997), sino también, como se ha podido comprobar en la “Sección 3”, por la calidad nutricional de las presas consumidas. La ausencia de diferencias en la respuesta inmune y en los valores isotópicos de las crías de las dos colonias consideradas indican que las diferencias observadas en las tasas de crecimiento de las crías en estas colonias deben atribuirse a una menor disponibilidad de alimentos y no a una menor calidad, lo que indicaría la existencia de una fuerte competencia entre las hembras lactantes por los recursos tróficos. Se podría argumentar que las dos colonias consideradas se hallan a sólo unos pocos kilómetros la una de la otra y que la influencia de la limitación de alimentos sobre el incremento de peso de las crías operaría a escalas espaciales mucho más grandes (Reid & Forcada, 2005). Sin embargo, los estudios de telemetría han demostrado que las hembras de león marino del norte (*Callorhinus ursinus*) de colonias colindantes usan diferentes áreas de alimentación, probablemente como resultado de ligeras diferencias en el orientación de la línea de costa

(Robson *et al*., 2004). Resultados similares se han observado entre colonias de león marino del sur separadas unas decenas de kilómetros (Campagna *et al*., 2001).

Independientemente de la razón por la cual la tasa de crecimiento corporal de las crías se veía afectada negativamente por el tamaño de la colonia, los datos obtenidos indican una relación negativa entre tasa de crecimiento de las crías y su tasa de supervivencia, así como entre la mortalidad y el tamaño de la colonia. Los depredadores pueden generar una densodependencia inversa cuando la respuesta funcional del depredador es asintótica (Gascoigne & Lipcius, 2004). Las crías de león marino del sur carecen de depredadores antes de entrar en el agua (Campagna *et al*., 1992), siendo el infanticidio la principal causa de muerte violenta para las crías de menos de cuatro semanas de edad (Campagna *et al*., 1992). Los datos aquí presentados indican que la tasa de supervivencia de las crías en tierra no sólo se correlaciona positivamente con el tamaño de la colonia, sino con la proporción de crías y machos subadultos. Además, la relación entre la tasa de supervivencia de las crías y la proporción de cría y machos subadulto se acerca a una curva de respuesta funcional de tipo II, un requisito previo para que un depredador pueda inducir densodependencia inversa. Como ha demostrado la presente tesis doctoral el número de machos subadultos en cada colonia varía ampliamente en toda la temporada de cría. Sin embargo, el número de crías por cada macho subadulto aumenta de forma dramática a lo largo de la temporada de cría debido al enorme aumento del número de crías, por lo que la supervivencia de las crías se incrementa simplemente por una dilución del riesgo (Krause & Ruxton, 2002).

Los motivos por los que los machos subadultos acosan y matan a las crías no se entienden completamente, pero se cree que es una conducta aberrante resultado de los esfuerzos para secuestrar y copular con las hembras custodiada por los machos adultos. Las incursiones, tanto en solitario como en grupo, constituyen la estrategia de apareamiento adoptada por los machos subadultos, incapaces de defender a un grupo de hembras en el centro de la colonia (Campagna *et al*., 1988a,b). Durante las incursiones, los machos adultos defienden ferozmente las hembras receptivas (Campagna & Le Bouef, 1988; Campagna *et al*., 1988a) y, en consecuencia y como alternativamente, los frustrados machos subadultos optan por raptar las crías y copular con ellas en la periferia de la colonia (Campagna et al. 1988a,b, 1992). Las hembras normalmente luchan para rescatar a

sus crías, pero tan pronto como salen de la zona central de la colonia para perseguir a sus crías secuestradas, son interceptadas por otros machos periféricos (Campagna *et al*., 1988b). Como resultado, los machos subadultos a menudo tienen éxito en el rapto de la cría, que posteriormente puede morir por el acoso sexual a que se ve sometida (Campagna *et al*., 1988b). Un punto importante a tener en cuenta es que el objetivo de los machos subadultos no es matar a las crías, sino copular con las hembras. En este contexto, el riesgo de que una cría sea raptada disminuiye rápidamente al aumentar el tamaño de la colonia, ya que los machos subadultos no las mataran proporcionalmente a su abundancia. Esta línea de razonamiento apoya los resultados aquí presentados y los publicados por Campagna *et al*. (1992), según los cuales la vulnerabilidad de las crías a los acosos por parte de machos subadultos es alta para unidades de cría aisladas y al inicio de la temporada de cría, pero disminuye rápidamente al aumentar el tamaño de la colonia, estabilizándose cuando la colonia supera el centenar de crías. Cabe destacar que las colonias con menos de 100 crías eran poco frecuentas en Argentina al inicio de la explotación comercial (Schiavini *et al*., 2004) pero predominaban tras el cese de la caza, cuando la tasa de crecimiento de la población era muy baja (Crespo & Pedraza, 1991; Dans *et al*., 2004). En cambio, la mayor parte de las colonias superaban dicho umbral a finales de los años ochenta, cuando la tasa de crecimiento de la población se aceleró (Crespo & Pedraza, 1991; Reyes *et al*., 1999; Dans *et al*., 2004; Grandi *et al*., 2008). Por el contrario, las colonias en las Islas Malvinas (Falkland Islands) a menudo albergan un número muy inferior a 100 crías (Thompson *et al*., 2005), por lo que cabría esperar una elevada tasa de mortalidad debido a las incursiones de los machos subadultos. De ser cierto esto último, explicaría por qué la población de las Malvinas no ha iniciado todavía su recuperación, tras el cese de la explotación comercial, hace casi cuarenta años. Sería necesario investigar en detalle la incidencia del infanticidio en dicha región para evaluar esta hipótesis.

Cambios históricos en la dieta del león marino del sur asociados a su explotación comercial y el desarrollo de la pesca industrial

Los datos presentados en los apartados anteriores han demostrado que la dieta del león marino del sur depende de su masa corporal y edad, así como también del estadio

reproductor en el caso de las hembras. Además, han demostrado la fuerte influencia de la dieta materna sobre la tasa de crecimiento de las crías durante las primeras semanas de vida, a pesar de que la tasa de mortalidad de las crías depende no sólo de su estado nutricional, sino también de la vulnerabilidad a los ataques por parte de machos subadultos. Cabe preguntarse ahora cómo ha variado la dieta del león marino del sur en respuesta a los cambios demográficos acontecidos durante el siglo XX, ya que las variaciones en el tamaño de la población provocadas por la caza habrían incrementado la disponibilidad de recursos *per capita*, al tiempo que el desarrollo de la pesca industria la habría reducido.

Se sabe que la reducción drástica de la población de león marino del sur debido a la caza comercial no fue el único impacto humano en el ecosistema marino en el norte de la Patagonia a lo largo del siglo XX, ya que en la década de los años setenta se inició la pesca industrial orientada principalmente a la captura de la merluza argentina (*Merluccius hubbsi*) (Crespo *et al*., 1997; Bertolotti *et al*., 2001). El desarrollo de la pesquería redujo drásticamente la población de merluza argentina (Lloris *et al*., 2003; Koen Alonso & Yodzis, 2005), lo que combinado con el pequeño tamaño de la población de león marino condujo a una severa reorganización del ecosistema (Koen Alonso & Yodzis, 2005), con grandes poblaciones de pingüinos de Magallanes *Spheniscus magellanicus* (Carribero *et al*., 1995; Schiavini *et al*., 2005), de calamar argentino (*Illex argentinus*) y de anchoa argentina (*Engraulis anchoita*) (Koen Alonso & Yodzis, 2005). Como consecuencia, algunos depredadores, como la mielga (*Squalus acanthias*), redujeron el consumo de merluza argentina e incrementaron el consumo de presas alternativas a medida que la abundancia de merluza declinaba (Koen Alonso *et al*., 2002).

Se sabe que la densidad de energía de las presas juega un papel central en la ecología trófica de al menos algunos otáridos (Rosen & Trites, 2000; Staniland *et al*., 2007) y que la densidad de energía de las presas pelágicas potenciales para los leones marinos es mayor que la de las presas bentónicas potenciales. Si la densidad de energía fuera el único criterio de elección de los leones marinos del sur, cabría esperar una preferencia por las presas pelágicas y un incremento de su consumo *per capita* entre 1940 y 1970, debido a la reducción de la competencia intraespecífica causada por la reducción del tamaño poblacional provocada por la caza comercial. Por el contrario, cabría esperar un

descenso en el consumo *per capita* de presas pelágicas a partir de 1970, debido a la disminución de la población de merluza argentina (*M. hubbsi*) derivada del desarrollo de la pesquería y a la recuperación parcial de la población de león marino.

Los datos isotópicos obtenidos durante la realización de la presente tesis doctoral no permiten una reconstrucción precisa de la composición de la dieta del león marino del sur entre 1940 y 2000, tal y como ya se ha mencionado, pues el factor de fraccionamiento de las presas al tejido óseo de esta especie se desconoce y los datos experimentales disponibles para otras especies de pinnípedos corresponden a otros tejidos (Hobson *et al*., 1996; Kurle, 2002). Sin embargo, los datos isotópicos obtenidos permiten detectar cambios en la contribución relativa de las presas bentónicas y pelágicas a la dieta de los leones marinos del sur a lo largo del siglo XX.

Si la contribución relativa de la merluza argentina (*M. hubbsi*) y otras presas pelágicas a la dieta de los leones marinos del sur hubiera disminuido en respuesta al desarrollo de la pesca industrial, el valor del $\delta^{13}C$ de los huesos del cráneo de los leones marinos debería haber aumentado. Sin embargo, los cambios observados en la señal del $\delta^{13}C$ del hueso siguen exactamente el patrón opuesto, por lo que la dieta actual debería considerarse como el resultado del cambio de una dieta basada en presas bentónicas en la década de 1970 a una dieta actual basada en un gran porcentaje de presas pelágicas, principalmente de merluza argentina. Además, la similitud entre las señales isotópicas de los huesos de los leones marinos varados en la década de 2000 y aquellos recogidos en los lugares donde fueron cazados en Península Valdés durante la década de 1940, indican dietas similares en ambos períodos.

El aumento de consumo *per capita* de la merluza argentina (*M. hubbsi*) y de otras especies de presas pelágicas por parte del león marino del sur en un momento en el que la población de merluza estaba en descenso resulta paradójico, pero puede ser explicado por tres mecanismos independientes y no excluyentes entre sí: (1) una mayor disponibilidad de juveniles de merluza debido a una reducción del canibalismo, (2) un aumento del consumo de merluza descartada por la pesquería y (3) una mayor explotación de las áreas de alimentación pelágicas debido al aumento de la competencia intraespecífica en las áreas de alimentación bentónicas costeras a medida que la población de león marino crecía.

El canibalismo es común en la merluza argentina (*M. hubbsi*) (Angelescu & Prenski, 1987), motivo por el cual la supervivencia de los individuos mas jóvenes podría aumentar si la pesca redujera el número de ejemplares de más edad (Laevastu & Favoritos, 1988). Este escenario es coherente con la estructura de la longitud de la población de merluza en la década de 1990, cuando los individuos de longitud menores de 30 cm dominaban la población (Bezzi *et al*., 2004), aunque no se sabe nada acerca de la longitud de la estructura de la población de merluza en la década de 1970.

Por otra parte, la pesca industrial dirigida a la merluza argentina (*M. hubbsi*) y al langostino (*Pleoticus muelleri*) descartó anualmente en el norte de la Patagonia alrededor de 170.000 toneladas de merluza argentina de talla inferior a los 30 cm durante la década de 1990 (Crespo *et al*., 1997; Cañete *et al*., 2000; Dato *et al*., 2003; Aubone *et al*., 2004). Este es precisamente el tamaño de merluza argentina preferido por parte de los leones marinos del sur (Crespo *et al*., 1997). Además, la biomasa total de merluza argentina descartada fue ligeramente superior a la biomasa que se espera que consuma anualmente la población de león marino del sur en la misma región (152.565 toneladas de acuerdo con Koen Alonso & Yodzis, 2005). Esto sugiere que, si bien los leones marinos del sur ciertamente no pueden localizar todas las merluzas descartadas, el descarte probablemente aumenta la disponibilidad de alimento para la especie.

La hipótesis de que los leones marinos del sur han aumentado el consumo de merluza argentina (*M. hubbsi*) en el norte de Patagonia debido a un incremento de la competencia intraespecífica por los recursos bentónicos costeros (Crespo & Pedraza, 1991) viene apoyada por los cambios opuestos en la señal isotópica y el tamaño poblacional experimentados desde la década de los años cuarenta. Un apoyo adicional proviene del mayor valor nutricional de las presas pelágicas, que puede compensar el desgaste energético derivado de viajes de alimentación más largos cuando se explotan áreas de alimentación pelágicas (Staniland *et al*., 2007). Una tercera línea de evidencia es la ausencia inesperada de diferencias entre los valores isotópicos de machos y hembras tanto en la década de 1940 como en la década de 2000, si bien existían en las décadas de 1970 y 1980. El león marino del sur es una especie altamente dimórfica, siendo los machos el doble en peso de las hembras (Cappozzo & Perrin, 2009), y como se ha mencionado anteriormente, el tiempo y la profundidad de buceo en pinnípedos dependen de las reservas

de oxígeno y, por tanto, de la masa corporal (Costa *et al*., 2004). Los datos aquí presentados concuerdan con una utilización más intensa de los recursos bentónicos por parte de los machos en comparación con las hembras, en las décadas de 1970 y 1990, pero no en las décadas de 1940 y 2000 cuando la señal isotópica de ambos sexos era indistinguible y coherente con una dieta basada en presas pelágicas por ambos sexos.

La comparación con los hábitos de alimentación de los leones marinos del sur de otras regiones pone de manifiesto la importancia de la pesca industrial en los cambios anteriormente indicados. La población chilena de leones marinos del sur también ha incrementado el uso de presas pelágicas en paralelo al desarrollo de una pesquería de cerco (Hückstädt & Antezana, 2003; Hückstädt *et al*., 2007), lo que ha resultado en una mayor accesibilidad a las presas pelágicas. Por el contrario, las señales isotópicas de los machos de leones marinos del sur de Tierra del Fuego, donde la pesca comercial es escasa, no ha cambiado en las últimas tres décadas, aunque esto debe ser considerado como una conclusión preliminar debido al pequeño tamaño y a la desigual distribución temporal de las muestras de dicha región analizadas en el contexto de la presente tesis doctoral. Sin embargo, la aparente ausencia de cambios refuerza la hipótesis de que el aumento del consumo de merluza argentina (*M. hubbsi*) en el norte de la Patagonia es un fenómeno local, causado paradójicamente por el desarrollo de la pesca industrial.

De todos modos, es necesario considerar una serie de factores que podrían influir en el cambio de la señal isotópica observado a lo largo del siglo XX en el norte de la Patagonia. El primero de esos factores es la invasión en la Provincia de Chubut por parte del alga bentónica *Undaria pinnatifida*, caracterizada por presentar valores bajos de $\delta^{13}C$. Esta especie, originaria del Pacífico, fue detectada por primera vez en Argentina en 1992 y actualmente es una de las especies dominantes en el conjunto de los macrófitos bentónicos intramareales de la provincia de Chubut (Casas *et al*., 2004). La llegada de *U. pinnatifida* a la costa del norte de la Patagonia podría haber causado un descenso en el valor de $\delta^{13}C$ de la toda la red trófica y, por tanto, una disminución en los valores del $\delta^{13}C$ de los cráneos de los leones marinos locales. Sin embargo, *U. pinnatifida* se registró por primera vez en la provincia de Chubut en 1992, mucho después del comienzo del declive de los valores $\delta^{13}C$ en los cráneos de los leones marinos del sur.

El segundo posible factor de confusión a considerar sería el efecto Suess, un cambio general en los reservorios de carbono de la superficie de los océanos provocado por la concentración cada vez mayor en el atmósfera de CO_2 empobrecido en ^{13}C como consecuencia de la combustión de combustibles fósiles. Este fenómeno ha sido invocado por Cullen *et al.* (2001) y Newsome *et al.* (2007) para explicar la disminución de los valores de $\delta^{13}C$ en varias especies del oeste del Golfo de Alaska y del este del Mar de Bering a lo largo de la segunda mitad del siglo XX, aunque otros autores argumentan que dicho cambio es debido a un descenso en la productividad del fitoplancton (Schell, 2000). Parece poco probable que el incremento de las emisiones de CO_2 empobrecido en ^{13}C haya sido el responsable de los cambios observados en el norte de la Patagonia, pues de ser así los valores de $\delta^{13}C$ de los cráneos de los leones marinos de Tierra del Fuego también deberían haber declinado, lo que no ha sucedido. Por otra parte, el conjunto de datos del norte de la Patagonia permite descartar un patrón de disminución constante en el $\delta^{13}C$ consistente con el efecto Suess, debido a que el $\delta^{13}C$ del hueso de los cráneos aumentó entre 1940 y 1970, para luego disminuir.

Los cambios cíclicos en la producción primaria en el Atlántico sudoccidental constituyen un tercer posible factor de confusión a tener en cuenta. La producción primaria en el Atlántico sudoccidental está influenciada por El Niño (ENSO) y por la rotación periódica del anticiclón subtropical (Venegas *et al.*, 1996; Fiedler, 2002). Una reducción en la producción primaria lleva asociado un descenso del $\delta^{13}C$ de los productores primarios y por lo tanto los valores isotópicos de toda la red trófica (Bidigare *et al.*, 1997). En consecuencia, la disminución de los valores del $\delta^{13}C$ de los huesos del cráneo de los leones marinos observada entre 1970 y 2000 podría simplemente reflejar la periodicidad de estos procesos climáticos si las muestras hubieran sido recolectadas en períodos de diferente productividad. Sin embargo, esta es una explicación poco probable, ya que el ENSO y el desplazamiento del anticiclón subtropical tienen una frecuencia cíclica de 4 y 15 años respectivamente, y el conjunto de datos de los cráneos del norte de la Patagonia abarca 30 años, con una distribución temporal de las muestras homogénea.

Por el contrario, la Oscilación Decadal del Pacífico (PDO) sí puede haber contribuido a la variabilidad observada, aunque los posibles vínculos entre PDO y la productividad en el Atlántico sudoccidental son poco conocidos. El período típico de la

PDO es de 20 a 30 años, y la última fase cálida duró desde el 1976 hasta el 2000 (Mantua & Hare, 2002), solapándose con el conjunto de datos del norte de la Patagonia (1974-2002). Por lo tanto, los leones marinos varados en el norte de la Patagonia antes de 1982 pueden haber experimentado los efectos de los últimos años de la fase fría del PDO (1947-1975) y aquellos varados en el 2001 y 2002 puede haber experimentado los efectos de una nueva fase fría sólo por un corto período antes de la muerte. Los huesos de leones marinos recogidos en el lugar de matanza de Península Valdés durante la década de 1940 también pudieron haber vivido la transición de una fase cálida (1925-1946) a un fase fría (1947-1975) (Mantua *et al*., 1997) y por tanto, podrían haber experimentado unas condiciones ambientales similares a las de los leones marinos varados en el norte de la Patagonia a finales de la década de 1990 y principios de la década de 2000. Sorprendentemente, los valores de $\delta^{13}C$ de los leones marinos recogidos en la década de 1940 y de aquellos varados al final de la década de 1990 y principios de la década de 2000 son similares entre sí y difieren de los de los leones marinos varados en la década de 1970. Además, el conjunto de datos del norte de la Patagonia abarca un periodo de constantes cambios en una serie de parámetros atmosféricos, como las anomalías de la temperatura del aire global, el índice de circulación atmosférica y el CO_2 atmosférico medido en el Mauna Loa (Chávez *et al*., 2003). Los cambios en estos parámetros son conocidos por estar relacionados con cambios multidecadales en la dinámica de los ecosistemas del Océano Pacífico (Chávez *et al*., 2003). Las anomalías de la temperatura del aire global y el índice de circulación atmosférica en la década de 1940 eran similares a aquellos de finales de la década de 1990, por lo que los cambios de la señal isotópica detectados en el norte de la Patagonia son compatibles con un posible cambio de régimen en el Atlántico sudoccidental relacionado con el cambio de régimen en el Océano Pacífico detectado por Chávez *et al*. (2003). Ahora bien, los datos de Tierra del Fuego no han revelado cambios importantes en la región y además el posible mecanismo causal de este hipotético cambio de régimen en el Atlántico sudoccidental se desconoce, sin que exista ninguna otra evidencia a su favor.

Como consecuencia, el aumento del consumo de presas pelágicas, principalmente de merluza argentina (*M. hubbsi*), por parte de los leones marinos del sur en la provincia de Chubut se mantiene como la hipótesis más probable para explicar los patrones de cambio isotópico aquí detectados.

Influencia de la pesca industrial sobre el crecimiento somático

Los datos expuestos en el apartado anterior indican que el desarrollo de la pesca industrial de la merluza en la Patagonia no parece haber reducido la disponibilidad de presas pelágicas para el león marino del sur. Al contrario, los datos indican un aumento de su consumo desde la década de 1970, lo que debería llevar aparejada una mejora de la calidad de la dieta, a tenor de la mayor calidad nutricional de las presas pelágicas expuesta anteriormente. Además, los resultados demuestran la gran capacidad por parte del león marino del sur para recuperar su función original en la red trófica a pesar de los profundos cambios y reorganización en el ecosistema causados por la actividad humana, ya que estos no parecen haber impedido la recuperación de la población del león marino del sur en el norte de Patagonia, cuya población es en la actualidad aproximadamente un tercio de su tamaño original antes de la explotación comercial y que durante los últimos años se ha incrementado de manera constante con una tasa de crecimiento anual próxima al 6% (Dans *et al*., 2004). La conclusión obvia de todo ello sería que desde la década de 1970, la población de león marino del sur presente en el norte de la Patagonia no ha experimentado una reducción de la disponibilidad de alimento *per capita*, a pesar de que la merluza argentina (*Merluccius hubbsi*) ha sido muy explotada por la pesca (Koen Alonso & Yodzis, 2005). La posible explicación a esta aparente paradoja se ha explicado en el apartado anterior.

Ahora bien, el análisis craneométrico realizado durante la presente tesis doctoral demuestra que si bien el crecimiento somático de los leones marinos del sur no cambió entre los años 1960 y 1990, desde entonces la talla media de los ejemplares adultos ha disminuido. Un patrón similar se ha observado en el oso marino del norte (*Callorhinus ursinus*), en el cual se sabe desde hace tiempo que el crecimiento somático y el tamaño de la población están inversamente correlacionados (Scheffer, 1955; Etnier, 2004).

La densodependencia del crecimiento somático viene, en última instancia, causada por una reducción *per capita* en la abundancia de las presas (Trites & Bigg, 1992) y por lo tanto, la reducción de las dimensiones cefálicas antes mencionadas indica que la mejora en la calidad de la dieta causada por un mayor consumo de especies pelágicas no ha

compensado la reducción de la disponibilidad *per capita* de presas a partir de la década de 1990. La disponibilidad de presas *per capita* para el león marino del sur puede haber disminuido debido a un incremento de su población, a una reducción de las poblaciones de sus presas principales (ej. merluzas) debido a la pesca, o una combinación de ambos factores. Tal como se ha visto anteriormente, las crías de leones marinos del sur en las grandes colonias crecen más lentamente que aquellas de colonias más pequeñas, probablemente debido a una menor disponibilidad de alimentos *per capita* para las madres durante la fase inicial de la lactancia, lo que sería una evidencia clara de densodependencia. El tamaño de las colonias ha aumentado conforme la población del león marino en el norte de la Patagonia se ha recuperado (Dans *et al*., 2004), lo que implicaría una reducción de la tasa de crecimiento somático de las crías aunque no existen datos históricos que permitan demostrarlo. Esto podría explicar la reducción del tamaño adulto, aunque parece poco probable que este sea debido únicamente a una mala alimentación durante la lactancia temprana debido a la existencia de crecimiento compensatorio en muchos mamíferos, incluidos los pinnípedos (Wilson & Osbourn, 1960; Worthy & Lavigne, 1983; Kamalzadeh *et al*., 1998; Eckhardt *et al*., 2005; Jeanniard du Dot *et al*., 2009). En este contexto, los resultados de un crecimiento reducido durante la lactancia podrían verse compensados por un crecimiento acelerado tras el destete si la cantidad de alimento lejos de la costa fuera elevada.

Por otra parte, la reducción del tamaño del cráneo es poco probable que sea el resultado de una pérdida de variabilidad genética, causada por la sobreexplotación comercial, ya que marcadores genéticos no han revelado ningún cuello de botella de la población (Feijoo M., datos no publicados). Además, la reducción del tamaño del cráneo, se inició en la década de 1990, aproximadamente tres décadas después del cese de la explotación comercial y después de que la población de león marino del sur comenzara a crecer (Crespo & Pedraza, 1991; Reyes *et al*., 1999), pero cuando el tamaño de la población era todavía pequeño en comparación con el existente antes de su explotación comercial (Crespo & Pedraza, 1991; Reyes *et al*., 1999). Cabe remarcar que, la reducción del tamaño del cráneo comenzó cuando los desembarques de merluza argentina (*M. hubbsi*) alcanzaron su punto álgido y el tamaño de la población de merluza estaba disminuyendo (Lloris *et al*., 2003; Koen Alonso & Yodzis, 2005), lo que indica que la

pesca industrial puede haber reducido la capacidad de carga del ecosistema para los leones marinos del sur en el norte de Patagonia.

El tamaño del cráneo está fuertemente correlacionado con el tamaño corporal en el león marino del sur (Rosas *et al*., 1993) y por lo tanto, una reducción en el tamaño del cráneo se espera que esté asociado a una reducción en el tamaño corporal. A su vez el tamaño del cuerpo está estrechamente vinculado a las habilidades de buceo en pinnípedos, incluido el león marino del sur tal como se explicó anteriormente. Esto sugiere que los hábitos de alimentación de los leones marinos del sur pueden haber cambiado como resultado de una disminución en el tamaño corporal causado por una menor disponibilidad de alimentos *per capita*. Los datos isotópicos indican que ambos sexos se han vuelto más pelágicos desde la década de 1970, de acuerdo con la respuesta esperada en caso de reducirse el tamaño corporal, aunque este resultado es también consistente con un mayor uso de presas de alta mar debido a un posible agotamiento de los recursos costeros causado por un aumento de la población de león marino y a un aumento del descarte por parte de la pesquería. Los datos isotópicos indican también que la dieta actual de los leones marinos del sur se acerca a la que se consumía antes del colapso de la población y que las diferencias de dieta entre machos y hembras están desapareciendo, lo que aumenta la competencia intraespecífica. Si este razonamiento es correcto y la pesca industrial ha reducido la capacidad de carga del ecosistema para los leones marinos en el norte de la Patagonia, la tasa de crecimiento de la población se espera que disminuya en un futuro próximo, a menos que la población de merluza vuelva a aumentar.

Para concluir, estos resultados indican que el estancamiento de la población del león marino una vez cesó su explotación comercial no puede atribuirse a una disminución de los recursos alimentarios, que sería en todo caso un fenómeno reciente. En cambio, consolidan la hipótesis de que la causa del estancamiento fue la reducción de tamaño de las colonias y su desestructuración social. En este contesto, habría aumentado la tasa de infanticidio debido a la presencia de una mayor proporción de machos subadultos no territoriales lo que debió dificultar el crecimiento de la población.

CONCLUSIONES

- La contribución de los recursos bentónicos a la dieta de los leones marinos del sur se incrementó en ambos sexos con la edad, siendo más elevada en los machos que en las hembras. Esto reflejaría que la obtención del alimento está estrechamente vinculada a la mejora de las habilidades de buceo como resultado de un aumento del tamaño corporal, aunque el aprendizaje podría jugar un papel una vez cesado el crecimiento.

- Los machos tuvieron una dieta más bentónica que las hembras cuando la población fue pequeña, pero al acercarse al tamaño original las diferencias se desvanecieron.

- El nivel trófico de los leones marinos es aproximadamente el mismo a lo largo de la ontogenia independiente del estadio de desarrollo y de su sexo. El elevado $\delta^{15}N$ en las hembras inmaduras se debe a la persistencia parcial de la señal de lactancia y no a un mayor nivel trófico.

- El valor nutricional de las presas potenciales pelágicas es superior al de las presas potenciales bentónicas.

- Las hembras incrementan el consumo de recursos bentónicos costeros después del parto probablemente para minimizar la duración de los viajes de alimentación y así el tiempo durante el cual las crías permanecen desatendidas. Sin embargo, esta estrategia podría comprometer el crecimiento de las crías, ya que una utilización intensiva de recursos bentónicos de menor calidad nutricional tiene un efecto negativo sobre la tasa de crecimiento de éstas.

- De lo anterior se deduce que las hembras se enfrentan al compromiso de escoger entre alimentarse de presas bentónicas costeras de menor calidad y pasar más tiempo con la cría, o alimentarse de presas pelágicas de mayor calidad y arriesgarse a perder la cría por causa de agresiones intraespecíficas.

- El tamaño de la colonia esta relacionado positivamente con la tasa de supervivencia de la cría y negativamente con la tasa de crecimiento, a pesar de que no existe diferencia en la composición de la dieta de las madres. La existencia de una relación inversa entre la tasa de crecimiento y la de supervivencia y el hecho de que la mortalidad de las crías es independiente del suministro de alimento indican que dicha mortalidad depende de otros factores, como el infanticidio.

- La importancia relativa de las presas pelágicas de mayor calidad nutricional ha aumentado en ambos sexos desde el desarrollo de la pesca industrial. Sin embargo, actualmente los ejemplares adultos son de menor talla que hace 30 años, lo que a su vez reduce la capacidad de buceo y los vuelve más dependientes de las presas pelágicas.

- De lo anterior se deduce que actualmente existe menos alimento disponible *per capita* que hace tres décadas. Esto sería debido no sólo al desarrollo de la pesca, sino también al propio crecimiento de la población de leones marinos, sin que la mejora de la calidad nutricional de la dieta haya podido paliar dicha reducción en la disponibilidad de alimento. Por lo tanto, es posible que la población de león marino se halle próxima a la capacidad actual de carga del ecosistema y no pueda alcanzar el tamaño de la población original.

- De todos modos, el estancamiento de la población del león marino del sur una vez cesó su explotación entre 1960 y 1980 no puede atribuirse a una disminución de los recursos alimentarios, ya que dicha disminución sería en todo caso un fenómeno reciente. Parece

probable que la causa del estancamiento fuera la reducción del tamaño y la desestructuración de las colonias de cría, lo que habría llevado aparejado un incremento en la tasa de mortalidad por infanticidio.

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Capítulo 1.
Incidencia de la edad, el sexo y el estado reproductivo sobre la dieta del león marino del sur

SECCIÓN 1.

CAMBIOS ONTOGÉNICOS EN LA DIETA DE LOS LEONES MARINOS DEL SUR

RESUMEN: Se analizaron los isótopos estables de carbono y nitrógeno de los huesos del cráneo de león marino del sur, *Otaria flavescens,* de la provincia de Chubut (Argentina) para determinar cómo cambian los hábitos de alimentación durante el desarrollo. El análisis de los isótopos estables mostró que el $\delta^{13}C$ aumentaba constantemente a lo largo del desarrollo de machos y hembras, excepto en los machos seniles en los cuales el $\delta^{13}C$ volvía a reducirse a un valor cercano al del estadio adulto primerizo. La comparación por pares de la proporción de isótopos estables del hueso en cada estadio de desarrollo puso de manifiesto diferencias entre machos y hembras sólo para los valores de $\delta^{13}C$ relativos a los estadios adulto primerizo y adulto. En general, los resultados indican que la contribución de las presas bentónicas a la dieta de ambos sexos aumenta con el estadio de desarrollo, excepto en los machos seniles, y que los machos adultos primerizos y machos adultos tienen una dieta más bentónica que las hembras en el mismo estadio de desarrollo, si bien no existen diferencias entre machos y hembras en los estadios de desarrollo de inmaduros y seniles. Con respecto al $\delta^{15}N$, la única diferencia observada radicaba en la señal isotópica de las hembras inmaduras, pues estaban más enriquecidas en ^{15}N que cualquier otro grupo. Esta diferencia se interpretó como el resultado de la persistencia de la señal de lactancia durante más tiempo en las hembras, al ser destetadas más tarde que los machos. En consecuencia, el nivel trófico de los leones marinos es aproximadamente el mismo en toda la vida, independiente del estadio de desarrollo y sexo. Por otra parte, el análisis de la curva de crecimiento mostró, para los dos sexos, diferencias estadísticamente significativas en la longitud condilobasal de los ejemplares inmaduros y los ejemplares adultos de los restantes estadios de desarrollo, indicando así que las diferencias en la dieta entre individuos en el estadio inmaduro y aquellos de los sucesivos estadios se deben probablemente a las diferencias en el tamaño corporal, mientras que las diferencias en la dieta entre los individuos de los últimos tres estadios podrían ser debidas a las diferentes habilidades de alimentación que se adquieren progresivamente durante su vida.

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Ontogenic dietary changes in South American sea lions

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Abstract

Stable isotopes of carbon and nitrogen in the skull bones of South American sea lions *Otaria flavescens* from the Chubut province (Argentina) were analysed to determine whether their feeding habits change during ontogeny. The stable isotope analysis showed that $\delta^{13}C$ steadily increased in males and females with their developmental stage (young, first adult, adult and senile), except in senile males whose $\delta^{13}C$ decreased to a value close to that of first adults. Pairwise comparison of bone stable isotope ratio in each developmental stage revealed differences between males and females only for the $\delta^{13}C$ values relative to first adults and adults. Overall, results indicate that the contribution of benthic prey items to the diet of both sexes increases with the developmental stage, except in senile males, and that first adult and adult males have a more benthic diet than females at the same developmental stage. No differences exist between males and females at younger and older developmental stages. With respect to $\delta^{15}N$, the only difference was in young female skulls, which were more enriched than those of any other group. Consequently, the trophic level of sea lions is roughly the same throughout life, independent of the developmental stage and sex, except for young females. The growth curve analysis revealed statistically significant differences in the condylobasal length of the skull between the sea lions in both sexes of the young stage and those of the other three developmental stages considered here but not among the individuals of the three later stages. This result indicates that the dietary differences between individuals in the young stage and those in the successive stages is likely due to differences in body size, whereas dietary differences among individuals of the later three stages might be due to different foraging skills that are progressively acquired during their life span.

Keywords: South American sea lion; *Otaria flavescens*; diet ontogeny; bone; stable isotopes.

Introduction

The foraging strategies of most air-breathing marine vertebrates are highly dependent on their diving skills, as they often forage underwater (Bjorndal, 1997; Berta & Sumich,

1999; Schreiber & Burger, 2001). Body mass is a critical parameter for diving vertebrates, not only because oxygen stores increase with body size (Schmidt-Nielsen, 1984; Gentry, Kooyman & Goebel, 1986; Kooyman, 1989; Costa, 1993; Fowler *et al*., 2007*b*) but also because of a faster use of oxygen stores in smaller species due to a higher mass-specific rate of metabolism (Brody, 1945; Miller & Irving, 1975; Ashwell-Erickson & Elsner, 1981; Schmidt-Nielsen, 1984; Thorson & Le Boeuf, 1994).

The influence of body size on diving skills is well exemplified in pinnipeds, as the adults of those species with a large body mass often exploit deep, benthic habitats, whereas the adults of species with a smaller body size typically behave as epipelagic predators (Gentry *et al*., 1986; Kooyman, 1989; Costa, 1991, 1993; Costa *et al*., 2004). Similarly, females of some highly dimorphic species have a more pelagic diet than males throughout their life, due to their smaller body size (Le Boeuf *et al*., 1996, 2000; Meynier *et al*., 2008). Nevertheless, the relationship between body size and habitat use is not univocal, as both sexes of some dimorphic species behave as pelagic predators (Costa *et al.*, 2004). This might be because animals with a better diving capacity choose to stay for longer in pelagic prey-rich patches instead of diving deeper or because a virtually non-existent continental shelf limits the availability of benthic habitats shallow enough to be accessed even by the best divers in the population (Hückstädt, Rojas & Antezana, 2007).

Experimental studies on the diving ontogeny of pinnipeds have also revealed a consistent increase in dive depth, dive time, travelling distance and swimming speed with age both in phocids (Lydersen, 1991; Lydersen & Hammill, 1993; Lydersen, Hammill & Kovacs, 1994; Thorson & Le Boeuf, 1994; Le Boeuf *et al*., 1996; Burns, 1999; Bekkby & Bjørge, 2000; Jørgensen *et al*., 2001) and otariids (Horning & Trillmich, 1997; McCafferty, Boyd & Taylor, 1998; Baker & Donohue, 2000; Baylis *et al*., 2005; Pitcher *et al*., 2005; Chilvers *et al*., 2006; Fowler *et al*., 2006; Spence-Bailey, Verrier & Arnould, 2007). This is probably because oxygen stores increase as the body mass of young pinnipeds increases during growth (Fowler *et al.*, 2006), but also because pinnipeds improve their control of oxygen demand with age (Rea & Costa, 1992; Ponganis, Kooyman & Castellini, 1993; Greaves *et al*., 2005; Fowler, Costa & Arnould, 2007*a*; Fowler *et al*., 2007*b*).

Improved diving skills with age are not expected to lead to an increase in the consumption of benthic prey items in obligate benthic foragers (e.g. Fowler *et al*., 2007*a*,*b*) or in oceanic species unlikely to reach the seabed (e.g., Lewis *et al*., 2006). Conversely, improved diving skills with age are expected to allow facultative foragers inhabiting the continental shelf to increase their access to the seabed and the time spent there as they grow, thus resulting in an increased contribution of benthic prey items to their diet (Le Boeuf *et al*., 1996). Unfortunately, ontogenic dietary changes in pinnipeds are poorly documented (Hobson & Sease, 1998; Newsome *et al*., 2006), and further research is needed to test the hypothesis that facultative foragers increase their consumption of benthic prey with increasing body size and age.

The South American sea lion *Otaria flavescens* is a highly dimorphic species, with adult males weighing 300-350 kg and adult females weighing 100-150 kg (Cappozzo, 2002). Both sexes have a pelagic diet in the eastern Pacific (Soto, Trites & Arias-Schreiber, 2006; Hückstädt *et al*., 2007), where pelagic prey occur at a high density; however, in the south-western Atlantic they are able to forage both close to the seabed and in the middle of the water column (Werner & Campagna, 1995; Thompson *et al*., 1998), with males exploiting deeper habitats than females (Campagna *et al*., 2001). Accordingly, mixed diets have been reported from northern Patagonia (Koen Alonso *et al*., 2000) and the Falkland (Malvinas) islands (Thompson *et al*., 1998). This dietary flexibility makes the South American sea lion a good subject to test the hypothesis that the consumption of benthic prey is related to body size and increases with age in facultative foragers.

Stable isotope analysis is a method especially well suited for this kind of study, as the stable isotope ratio in the consumer's tissues integrates the stable isotope ratio of its prey items in a predictable manner over a long period of time (DeNiro & Epstein, 1978, 1981). Stable isotope analysis offers less detailed information on dietary composition than scat analysis; however, because it provides information on assimilated food, it has become a standard method in the study of the foraging ecology of pinnipeds (Aurioles, Koch & Le Boeuf, 2006; Newsome *et al.*, 2006; Hückstädt *et al.*, 2007; Ducatez *et al.*, 2008; Porras-Peters *et al.*, 2008). The ratio $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) increases with each trophic level due to the preferential excretion of the lighter stable isotope (Minagawa & Wada, 1984) and, therefore, is used to assess the trophic level (McCutchan *et al.*, 2003). Differences in

the stable carbon isotope ratios between the pelagic and benthic primary producers are usually much larger than those observed among trophic levels (Cardona *et al.*, 2007); therefore, $\delta^{13}C$ is often used to discriminate between habitats, because benthic species are usually enriched in ^{13}C relative to pelagic species. Consequently, stable isotopes cannot be used to determine the depth of the foraging habitat but do allow for differentiating benthic from epipelagic predators by analysing a tiny tissue sample.

Following this reasoning, here we analyse stable carbon and nitrogen isotopes in skull bone fragments from the South American sea lion to test three predictions emerging from the hypothesis that the consumption of benthic prey items is related to body size and age in facultative foragers: 1) the consumption of benthic prey items will increase from post-weaning to adulthood within each sex; 2) adult females will have a more pelagic diet than adult males; 3) the consumption of benthic prey items will also increase with age in non-growing adults.

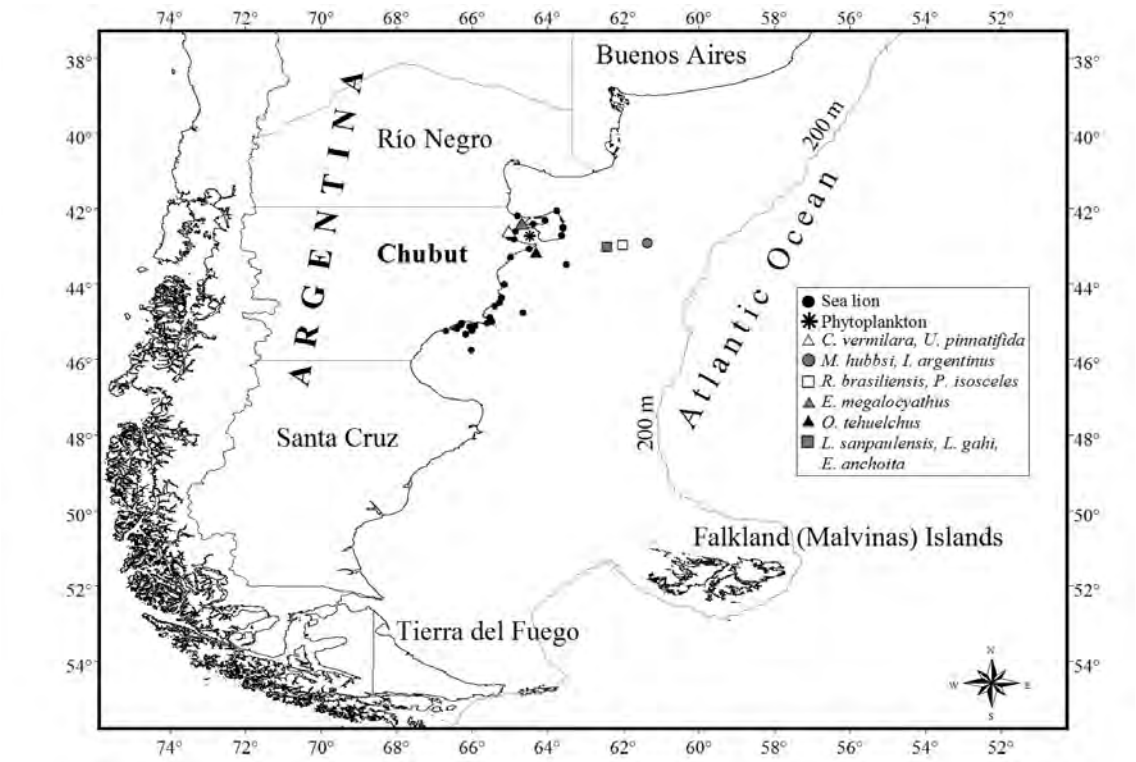

Figure 1 Atlantic coast of Argentina and location of the Chubut province. Symbols show the sampling location of primary producers, South American sea lions *Otaria flavescens* and potential prey.

Materials and methods

Sampling

Samples (40 males and 40 females for stable isotope analyses and 116 males and 84 females for the length-age relationship) were collected from sea lion skulls of the scientific collection of Centro Nacional Patagonico (CENPAT) at Puerto Madryn, Argentina. Skulls selected for sampling were restricted to sea lions that were incidentally captured by fishermen or stranded and died along the coast off the Chubut province in northern Patagonia (Fig. 1) from 1990 to 2000 to avoid the possible confounding effects of long-term changes in growth rate and/or stable isotope baseline. The age of all the sampled individuals had previously been assessed by counting growth layers in the dentine of the canines (Crespo, 1988; Crespo *et al*., 1994), and the age ranged from 1 year to 19 years for stable isotope analysis (Table 1) and from 1 month to 20 years for the length-age relationship analysis (Fig. 2).

The life span of South American sea lions is *c.* 18-20 years (King, 1983; Crespo, 1988), with females reaching adulthood at about 4 years of age and males at about 6 years of age (Vaz-Ferreira, 1981; Cappozzo, 2002). Based on these data, we established four developmental stages (young, first adult, adult and senile). Accordingly, we selected the following from the above-mentioned skulls to analyse the $\delta^{13}C$ and $\delta^{15}N$ in the bone: 10 young individuals of each sex (post-weaned and not yet sexually mature individuals between 1 and 5 years of age for males and between 1 and 3 years of age for females), 10 first adult individuals of each sex (sexually mature individuals between 7 and 8 years of age for males and between 5 and 7 years of age for females), 10 adult individuals of each sex (sexually mature individuals between 9 and 12 years of age for males and between 8 and 12 years of age for females) and 10 senile individuals of each sex (sexually mature individuals >12 years old). The main difference between first adults and adults is that the former can still grow in length, whereas the latter are thought to have ceased length growth. The term subadult was not used because it implies that the individual is not sexually mature.

The skulls were first cleaned using dermestid beetles, washed with water and soap, thoroughly rinsed with water and air dried. A small fragment of turbinate bones from the nasal cavity was collected from each skull. This bone type was selected because it is easy

to crush and its sampling does not damage the skull for subsequent studies. The condylobasal length of each skull was also measured, as it is highly correlated with

Table 1 Stable isotope values of the South American sea lion *Otaria flavescens* skull sample from Chubut province investigated in this study: age (in years) and sampling date (year of collection)

	♂				♀			
Developmental stage	Age	Sampling date	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Age	Sampling date	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Young	1	1993	-13.0	24.3	1	1998	-13.3	22.7
	1	1993	-12.8	26.2	1	1996	-12.6	23.9
	1	1995	-13.5	24.5	1	1999	-12.9	25.5
	2	1994	-13.1	23.2	1	1993	-13.0	24.8
	3	1994	-13.1	21.1	1	1999	-12.9	25.9
	3	1991	-12.9	20.9	1	1995	-14.8	25.3
	3	1994	-13.6	19.9	2	1998	-14.4	24.3
	5	1997	-13.1	21.1	3	1996	-12.7	22.5
	5	2000	-12.7	21.3	3	1991	-12.4	23.1
	5	2000	-12.6	21.9	3	1990	-12.4	21.9
First adult	7	1991	-11.3	24.1	5	2000	-13.5	20.9
	7	1992	-12.1	21.8	5	2000	-13.0	20.1
	7	2000	-11.7	21.5	5	1994	-12.9	21.7
	7	1994	-12.8	21.7	6	1993	-12.6	23.3
	7	2000	-12.1	22.0	6	1993	-12.7	22.8
	8	1994	-12.5	22.7	6	2000	-13.1	21.0
	8	1997	-12.9	21.9	7	1991	-12.3	22.1
	8	1998	-12.3	22.7	7	1992	-13.0	21.9
	8	2000	-10.7	21.6	7	1990	-11.6	22.4
	8	2000	-12.7	21.7	7	1996	-13.3	21.7
Adult	9	1999	-11.4	22.9	8	1990	-12.3	22.2
	9	1990	-11.4	19.1	8	1994	-12.1	22.2
	9	1993	-11.9	23.2	9	1993	-12.0	23.3
	10	1994	-11.3	22.9	9	2000	-13.0	22.4
	10	1993	-11.6	23.4	9	1994	-12.0	22.6
	10	2000	-10.9	23.5	10	1995	-11.8	22.4
	11	1996	-11.2	22.3	10	2000	-12.6	22.0
	11	1990	-11.6	22.0	11	1994	-12.3	22.3
	12	1990	-11.9	23.0	12	1995	-12.5	21.8
	12	1990	-11.0	22.4	12	1990	-13.1	21.6
Senile	13	1994	-11.5	22.5	13	1996	-11.6	22.0
	13	2000	-12.5	22.1	13	1996	-11.5	23.4
	13	2000	-12.5	21.5	13	1990	-11.7	22.2
	13	2000	-11.4	22.3	14	1993	-11.5	23.3
	16	1998	-11.5	22.2	15	1995	-12.5	21.6
	16	1994	-12.3	21.0	15	2000	-11.1	23.2
	16	2000	-12.4	22.9	16	1998	-12.1	22.3
	17	1995	-11.8	22.6	17	2000	-12.1	23.0
	18	1998	-11.6	22.3	18	1994	-12.3	22.0
	19	2000	-12.6	21.1	19	2000	-11.8	22.6

standard length in South American sea lions (Rosas, Haimovici & Pinedo, 1993) and can thus be used as a proxy for body length. The condylobasal length was also measured in additional skulls in order to obtain a larger sample to study the length-age relationship.

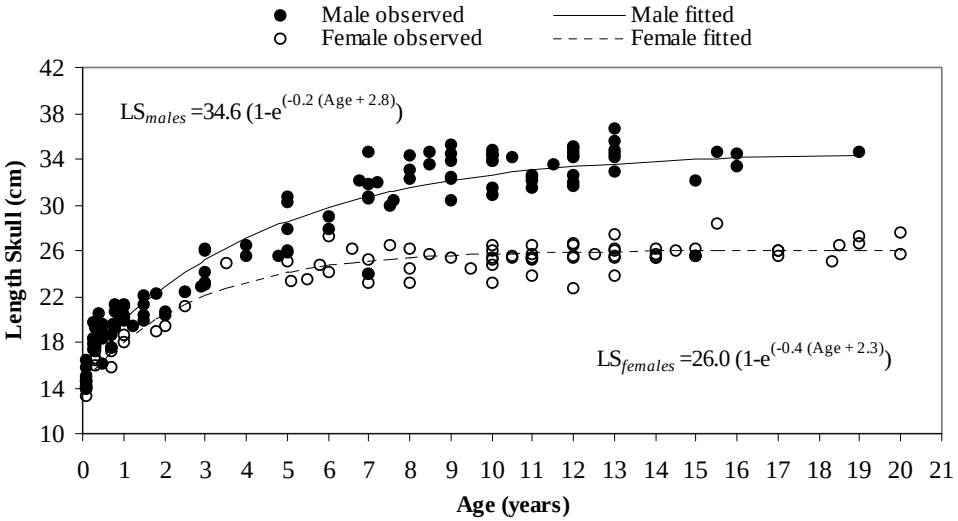

Figure 2 Growth curves of the male and female South American sea lions *Otaria flavescens* from Chubut province, calculated according to the von Bertalanffy equation. Sample size: n = 116 for male and n = 84 for female.

Samples of the most important potential prey species for South American sea lions off the Chubut province (Koen Alonso *et al.*, 2000) were collected in January and February 2006 (Table 2). Pelagic species were represented by Argentine short-fin squid *Illex argentinus*, Argentine anchovy *Engraulis anchoita*, South-American long-fin squid *Loligo sanpaulensis*, Argentine hake *Merluccius hubbsi* and Patagonian squid *Loligo gahi*, whereas benthic species were represented by red octopus *Enteroctopus megalocyathus*, tehuelche octopus *Octopus tehuelchus*, flounder *Paralichthys isosceles* and banded cusk eel *Raneya brasiliensis*. White muscle and mantle were collected from fish and cephalopods, respectively. Samples of benthic primary producers (*Codium vermilara* and *Undaria pinnatifida*) and phytoplankton (diatoms and dinoflagellates) were also collected to determine their stable isotope ratios and to produce a complete and better description of the isotopic landscape off the Chubut province. The samples of the potential prey items and primary producers were provided by local fishermen or collected by the staff of the

Table 2 Mean and standard deviation of the stable isotope values of primary producers and potential prey of the South American sea lion *Otaria flavescens* off Chubut province (northern Patagonia, Argentina).

Scientific name	Common name	Habitat	Sampling date	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Primary Producers						
Phytoplankton		P	January 2006	2[a]	-21.0 ± 0.1	13.4 ± 0.6
Seaweed						
Codium vermilara		B	January 2006	5	-14.9 ± 1.3	11.6 ± 0.4
Undaria pinnatifida	wakame	B	January 2006	5	-19.1 ± 1.2	10.4 ± 0.5
Potential Prey						
Fish						
Merluccius hubbsi (> 30 cm)	Argentine hake	P	February 2006	5	-17.0 ± 0.5	17.1 ± 0.4
Merluccius hubbsi (< 30 cm)	Argentine hake	P	February 2006	5	-17.7 ± 0.6	15.9 ± 0.5
Engraulis anchoita	Argentine anchovy	P	January 2006	5	-17.9 ± 0.2	15.7 ± 0.8
Paralichthys isosceles	Flounder	B	January 2006	5	-15.9 ± 0.4	18.0 ± 0.6
Raneya brasiliensis	Banded cusk eel	B	January 2006	5	-15.3 ± 0.6	18.8 ± 0.5
Cephalopod						
Illex argentinus	Argentine shortfin squid	P	February 2006	5	-17.0 ± 0.6	13.7 ± 0.8
Loligo sanpaulensis	South American longfin squid	P	January 2006	5	-16.8 ± 0.2	17.2 ± 0.3
Loligo gahi	Patagonian squid	P	January 2006	4	-17.6 ± 0.4	15.7 ± 0.6
Enteroctopus megalocyathus	Red octopus	B	February 2006	5	-14.6 ± 0.7	18.9 ± 0.9
Octopus tehuelchus	Tehuelche octopus	B	February 2006	5	-14.8 ± 0.2	19.9 ± 0.4
Benthic prey[b]				4	-15.1 ± 0.6	18.9 ± 0.8
Pelagic prey[c]				6	-17.3 ± 0.5	15.9 ± 1.3

[a]Collective samples of diatoms and dinoflagellates, including several individuals.

[b]Gross mean of the benthic prey. Sample size refers to the number of species.

[c]Gross mean of the pelagic prey. Sample size refers to the number of species.

Habitat type: pelagic (P), benthic (B); Sample size (*n*)

Laboratory of Marine Mammals of CENPAT. Phytoplankton was collected with a 20-μm mesh-size plankton net and, once in the laboratory, filtered in a pre-combusted GF/C filter.

All skull bone samples were stored dry, whereas all potential prey items and primary producer samples were stored in a freezer at -20ºC.

Stable isotope analysis

Following initial sampling, cleaning and preparation, the skull bone fragments and the other samples (primary producers and potential prey items) were dried at 60ºC and ground into a fine powder using a mortar and pestle. Lipids were extracted with a chloroform/methanol (2:1) solution (Bligh & Dyer, 1959), because lipids are depleted in

^{13}C compared with other molecules, thus confounding the results by decreasing the $\delta^{13}C$ value (DeNiro & Epstein, 1977; Tieszen *et al*., 1983). Bone and phytoplankton samples contain a high concentration of inorganic carbon that may add undesirable variability to $\delta^{13}C$ (Lorrain *et al*., 2003). Consequently, they were previously treated by soaking for 24 h in 0.5 N (bone) and 0.05 N (phytoplankton) hydrochloric acid (HCl) to decarbonise them (Ogawa & Ogura, 1997; Newsome *et al*., 2006). Because HCl treatment adversely affects $\delta^{15}N$ (Bunn, Loneragan & Kempster, 1995), each of the samples was divided into two sub-samples, respectively, used for C analyses after decarbonation and for N analyses without decarbonation.

Approximately 1 mg of dried bone, 4 mg of homogenized seaweeds, 16 mg of homogenized phytoplankton with filter and 0.6 mg of white muscle from fish and of mantle from cephalopods were weighed into tin cups (3.3 x 5 mm), combusted at 900ºC and analysed in a continuous flow stable isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses. Samples were processed at Scientific-Technical Services of the University of Barcelona.

Stable isotope abundances, expressed in delta (δ) notation, where the relative variations of stable isotope ratios are expressed in permil (‰) deviations from pre-defined international standards, were calculated as:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N, R_{sample} is the heavy-light stable isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and $R_{standard}$ is the heavy-light stable isotope ratio in reference standards, which were V-PDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and the atmospheric nitrogen (air) for ^{15}N. International stable isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the International Atomic Energy Agency (IAEA, Vienna), namely polyethylene (IAEA CH_7, $\delta^{13}C$ = -31.8 ‰), graphite (IAEA $USGS_{24}$, $\delta^{13}C$ = -16.1 ‰) and sucrose (IAEA CH_6, $\delta^{13}C$ = -10.4 ‰), were used for calibration at a precision of 0.2 ‰. For nitrogen, the international stable isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N_1, $\delta^{15}N$ = +0.4 ‰ and IAEA N_2, $\delta^{15}N$ = +20.3 ‰) and KNO_3 (IAEA NO_3, $\delta^{15}N$ = +4.7 ‰), were used to a precision of 0.3 ‰.

Data analyses

The length-age relationship was calculated according to the von Bertalanffy growth curve:

$$L_t = L_\infty (1 - \exp [- K (t - t_0)])$$

where L_t is the length at age t, L_∞ is the theoretical maximum (or asymptotic) length that individuals would reach if lived indefinitely, K is a growth coefficient and t_0 is the theoretical age at zero length.

One-way ANOVA, followed by the Scheffe *post hoc* test, was used to detect differences in the skull length of the individuals of each sex in each developmental stage (young, first adult, adult and senile), because the sample size for condylobasal length was not constant. Nested ANOVA was used to test the influence of habitat type (benthic *vs.* pelagic) and species identity on the $\delta^{13}C$ and $\delta^{15}N$ of prey species. Two-way ANOVA was used to assess the effect of sex and developmental stage in bone stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$). One-way ANOVA, followed by Tukey *post hoc* test, was later used to compare bone stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of different developmental stages within each sex. Furthermore, Student's *t*-test was used to investigate differences in bone stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of both sexes in each different developmental stage.

Data are shown as mean ± standard deviation, unless otherwise stated. All statistical analyses were conducted with the SPSS 15 software package. Before all other analyses, normality was tested by means of the Lilliefors test and homoskedasticity by means of the Levene test.

Results

The von Bertalanffy growth curves for both sexes (Fig. 2) confirmed the marked sexual dimorphism of the South American sea lion, as males had an asymptotic condylobasal length of 34.6 cm, whereas that of females was 26.0 cm. Furthermore, the period of fast growth before the adulthood stasis was longer in males (7-8 years) than in females (5-7 years). Nevertheless, in both sexes there was no measurable skull growth after the sexual maturity. As a consequence, one-way ANOVA (females: $F_{3,79} = 158.206$, $P < 0.001$; males: $F_{3,110} = 159.785$, $P < 0.001$) and a Scheffe *post hoc* test (Fig. 3) revealed that the

skull length of young sea lions of each sex was significantly smaller than that of sea lions at the other three developmental stages here considered, whereas no statistically significant difference was found among the individuals of each sex in the first adult, adult and senile developmental stages.

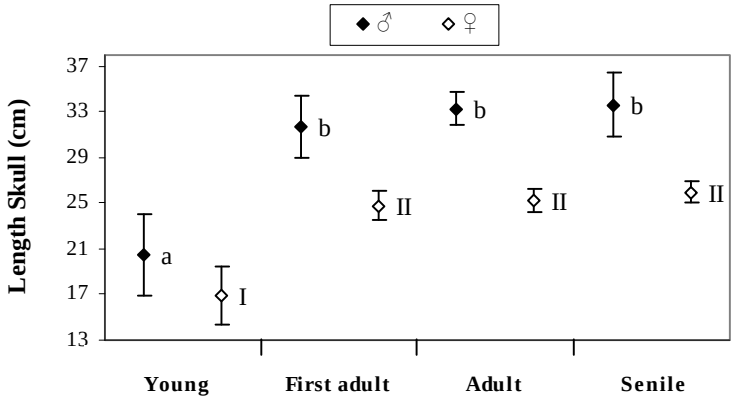

Figure 3 Mean and standard deviation of the skull length of South American sea lions *Otaria flavescens* at the four developmental stages. Developmental stages within each sex with different letters (males) or Roman numerals (females) differ in their mean values. Sample size: n = 60 for young males, n = 14 for first adult males, n = 28 for adult males, n = 12 for senile males, n = 20 for young females, n = 11 for first adult females, n = 26 for adult females and n = 26 for senile females.

The $\delta^{13}C$ values of the primary producers ranged from -14.9 ± 1.3 ‰ for seaweeds to -21 ± 0.1 ‰ for phytoplankton, whereas those of the potential prey items ranged from -14.6 ± 0.7 ‰ for benthic species, like the red octopus, to -17.9 ± 0.2 ‰ for pelagic species, like the Argentine anchovy (Table 2 and Fig. 4). Accordingly, benthic potential prey species ($\delta^{13}C$: -15.1 ± 0.6 ‰; $\delta^{15}N$: 18.9 ± 0.8 ‰) were, on average, more enriched both in ^{13}C (nested ANOVA: $F_{9,39}$ = 235.170, P < 0.001) and ^{15}N (nested ANOVA: $F_{9,39}$ = 318.709, P < 0.001) than pelagic species ($\delta^{13}C$: -17.3 ± 0.5 ‰; $\delta^{15}N$: 15.9 ± 1.3 ‰), although the $\delta^{15}N$ primarily increased with trophic level (Fig. 4).

The $\delta^{13}C$ of skull bone ranged from -13.6 to -10.7 ‰ for males and from -14.8 to -11.1 ‰ for females, whereas the $\delta^{15}N$ of skull bone ranged from 19.1 to 26.2 ‰ for males and from 20.1 to 25.9 ‰ for females (Table 1). The average $\delta^{13}C$ of skull bone ranged from -13.0 ± 0.3 ‰ in young males to -11.4 ± 0.3 ‰ in adult males and from -13.1 ± 0.8 ‰ in young females to -11.8 ± 0.4 ‰ in senile females. The average $\delta^{15}N$ of skull bone ranged from 22.0 ± 0.6 ‰ in senile males to 22.5 ± 1.3 ‰ in adult males and from 21.8 ± 1.0 ‰ in

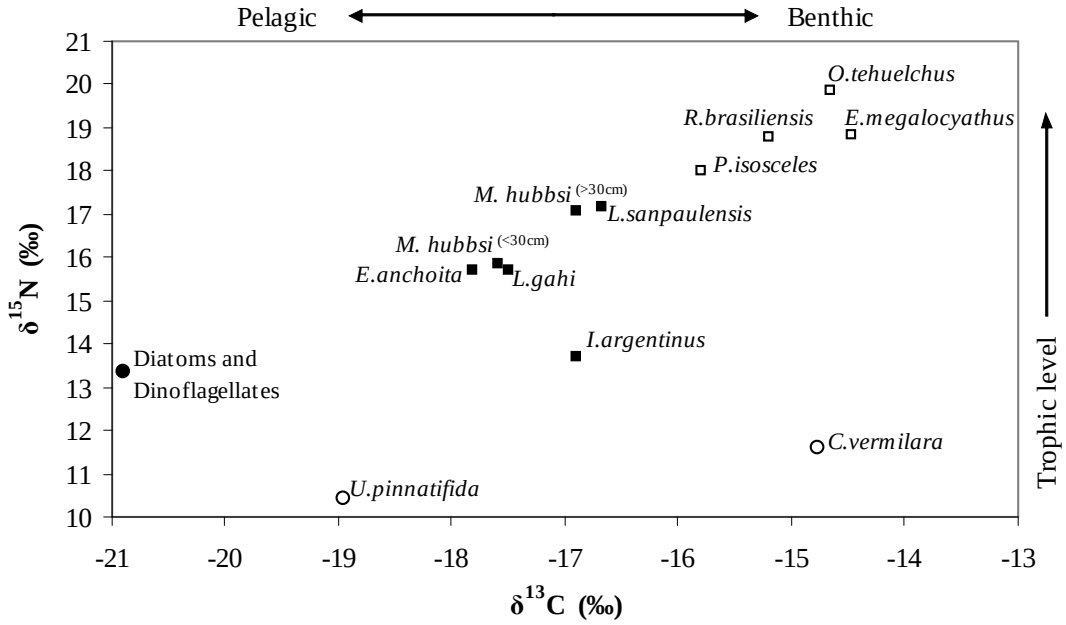

Figure 4 Mean bivariated stable isotope values of the primary producers and the main prey of the South American sea lion *Otaria flavescens* off the Chubut province. Primary producers: seaweeds (○) and phytoplankton (●). Main prey: benthic species (□) and pelagic species (■). Sample size: $n = 5$ for all the species, except for the *Loligo gahi* ($n = 4$) and phytoplankton ($n = 2$; collective samples).

first adult females to 24.0 ± 1.4 ‰ in young females (Fig. 5). Two-way ANOVA (sex x developmental stage) revealed statistically significant differences in the average $\delta^{13}C$ of males and females ($F_{7,72} = 10.477$, $P = 0.002$) and also among the average $\delta^{13}C$ of the developmental stages ($F_{7,72} = 22.196$, $P < 0.001$). As well, there was a statistically significant interaction term ($F_{7,72} = 4.791$, $P = 0.004$), thus pointing out that the pattern of the ontogenetic change of the $\delta^{13}C$ is not the same in both sexes. This is because the $\delta^{13}C$ of both females and males increased steadily with the developmental stage, except in senile males, whose $\delta^{13}C$ decreased to a value close to that of first adults according to the Tukey *post hoc* test (Fig. 5). Pairwise comparison of the bone $\delta^{13}C$ values of males and females in the same developmental stage revealed differences for first adults and adults but not for young and senile individuals (Fig. 5).

Two-way ANOVA (sex x developmental stage) revealed statistically significant differences in $\delta^{15}N$ among the developmental stages considered ($F_{7,72} = 4.354$, $P = 0.007$) without statistically significant differences between sexes ($F_{7,72} = 2.180$, $P = 0.144$) but with a statistically significant interaction term ($F_{7,72} = 2.996$, $P = 0.036$). Such differences

were because the skulls of young females were more enriched in ^{15}N than those of the individuals of the other three developmental stages (Fig. 5), as revealed by the Tukey *post hoc* test. As a consequence, the trophic level of sea lions remained roughly the same throughout their life, independent of developmental stage and sex, with the exception of young females (Fig. 5).

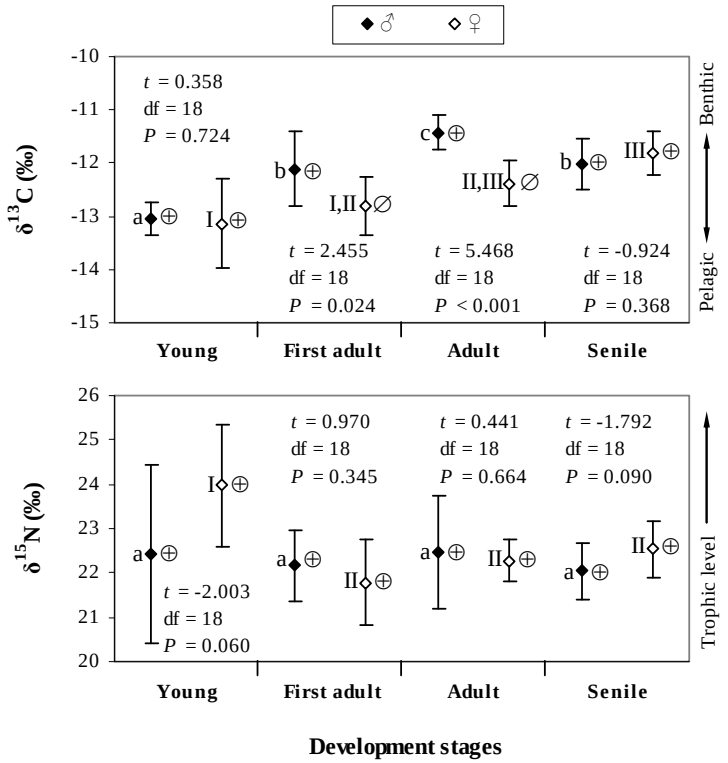

Figure 5 Mean and standard deviation of the bone $\delta^{13}C$ and $\delta^{15}N$ of males and females at the four developmental stages. Developmental stages of each sex with different letters (males) or Roman numerals (females) differ in their mean values according to Tukey *post hoc* test. The summary statistics of the corresponding ANOVA are shown in the text. Males and females of each developmental stage with a different symbol (⊕ *vs.* ∅) differ in their mean values according to Student's *t* test. Sample size: $n = 10$ for each sex and developmental stage.

Discussion

The isotopic data presented here do not allow for a precise reconstruction of the composition of the diet of South American sea lions, because prey-to-predator bone fractionation factors have not been experimentally determined for this species and those determined experimentally for other pinniped species are for tissues other than bone (Hobson *et al.*, 1996; Kurle, 2002). However, the stable isotope data presented here are

useful for detecting changes in the relative contribution of benthic and pelagic prey items to the diet of South American sea lions, as long as the $\delta^{13}C$ and $\delta^{15}N$ gradients in the local ecosystem have not been inverted from the time the sea lions were stranded (1990-2000) and when the samples of the potential prey species were collected (2006). More precisely, the stable isotope landscape off the Chubut province indicates that changes in the $\delta^{13}C$ of the bone material should primarily reflect changes in the proportion of benthic and pelagic prey items in the diet, whereas changes in the $\delta^{15}N$ should be primarily linked to changes in trophic level.

Results suggest that consumption of benthic prey items increased from post-weaning to adulthood in both sexes, except in senile males, thus supporting the hypothesis that the early foraging ontogeny of the South American sea lion is tightly linked to the improvement of diving skills resulting from an increase in body mass (Le Boeuf *et al*., 1996; Horning & Trillmich, 1997). Differences in body mass explain not only why diet changed with age, but also, why first adult males and adult males had a much more benthic diet than females of the same developmental stage.

However, this interpretation of the ontogenic changes in the stable isotope ratio of skull bone is highly dependent on the actual rate of bone turnover and discrimination between age classes (Tieszen *et al*., 1983; Hobson & Clark, 1992; Hobson, 1993). Suckling pinniped pups are more enriched in ^{15}N than their mothers, whereas the relationship between suckling pups and their mothers is less clear for ^{13}C and may be species dependent (Newsome *et al.*, 2006; Ducatez *et al.*, 2008). Experimental evidence indicates that suckling pups of the South American sea lion are more enriched in ^{15}N than their mothers are, without any significant mother-to-pup fractionation for carbon (M. Drago, unpubl. data). At least in other pinniped species, the suckling signal decays after weaning and is completely turned over from bone 10 months later (Newsome *et al.*, 2006). Conversely, bone turnover is likely to be substantially slower in the other age classes and hence stable isotope ratios in bone may be much less discernable between the older age classes than between young and first adult sea lions.

The persistence of the suckling signal may have affected the $\delta^{15}N$ values of some of the young individuals analysed here, because South American sea lions are weaned when they are 6-10 months old, with males being weaned earlier than females (Crespo, 1988).

Consequently, South American sea lions older than 20 months (1.8 years) are expected to have completely turned over the suckling signal, with the stable isotopic ratios of younger individuals being a mixture of the suckling signal and that of their own diet. Only three of the 10 young males included in the present study were younger than 2 years and, hence, susceptible of exhibiting traces of the suckling signal, but up to six young females may have exhibited it. The persistence of some traces of the suckling signal in some of the young analysed probably explains why the standard deviation of the $\delta^{15}N$ of young sea lions of each sex was larger than that of other developmental stages and why the $\delta^{15}N$ of young females was higher than that of the females in other developmental stages. However, the interpretation of the ontogenic changes in the diving skills of the South American sea lions is derived from the $\delta^{13}C$ data, not affected by the persistence of traces of the suckling signal.

Length growth may explain increased consumption of benthic prey items during the early ontogeny of each sex and the differences between sexes, but the consumption of benthic prey items continued to increase steadily in both sexes after puberty, although South American sea lions have determinate length growth (Rosas *et al.*, 1993; present study). Consequently, continued improvement of diving skills through life cannot be explained only by length growth. Nothing is known about weight gain in the South American sea lion, but weight stabilizes after puberty much later than length in Steller sea lions *Eumetopias jubatus* (Winship, Trites & Calkins, 2001). Weight also increases with age in adult female New Zealand sea lions *Phocarctos hookeri* that range from 10 to 17 years old, although age explains only 7% of the weight variability (Chilvers *et al.*, 2006).

A similar weight-age pattern in South American sea lions may explain the increased access to benthic prey items of the first adult, adult and senile individuals as compared with young individuals. However, it is unlikely that it explains the steady increase in the consumption of benthic prey items in females after puberty, considering the high variability in the body weight of adult sea lions of other species (Winship *et al.*, 2001; Chilvers *et al.*, 2006), the absence of a correlation between age and dive depth in adult female New Zealand sea lions (Chilvers *et al.*, 2006) and the wide weight variability expected for female South American sea lions in agreement with the length variability (Rosas *et al.*, 1993; present study).

We believe that increased access to benthic prey items with age after puberty cannot be explained only by weight growth, although bigger individuals can dive deeper. Young pinnipeds have limited access to deep, benthic resources, not only because they have smaller oxygen stores but also because they appear unable to regulate heart rate, respiration, vasoconstriction or body temperature as effectively as adults (Rea & Costa, 1992; Ponganis *et al*., 1993; Greaves *et al*., 2005; Fowler *et al*., 2007*a*,*b*). As a consequence, young pinnipeds rely on energy produced anaerobically after long/deep dives more often than adults do (Kooyman *et al*., 1983; Burns, 1999), although adult pinnipeds foraging on the seabed often exceed their calculated aerobic dive limit (Costa & Gales, 2000, 2003; Costa, Gales & Goebel, 2001; Chilvers *et al*., 2006). This is because tight control of the cardiorespiratory function allows adult pinnipeds to significantly reduce their rate of metabolism while submerged (Hurley & Costa, 2001), thus enabling them to have access to larger and more rewarding prey items than those available for epipelagic foragers and also reducing competition with younger individuals (Costa & Gales, 2003; Page, McKenzie & Goldsworthy, 2005).

We hypothesize that increased consumption of benthic prey items with age might depend on age-related learning. Furthermore, if improvement of those skills continues through life, pinnipeds are expected to increase dive depth and dive time with age, even after reaching final adult body size. The reduced consumption of benthic prey items by senile males is, however, intriguing and suggests a decrease in diving performance, perhaps due to a physiological debilitation caused by a very high energetic investment in breeding (Campagna & Le Boeuf, 1988). Such a hypothesis is supported by a mortality peak for 11-year-old males (Crespo, 1988) and also by a possible reduction in body weight, as reported for the Steller sea lion (Winship *et al*., 2001). However, weight-at-age data are urgently needed to test this hypothesis.

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SECCIÓN 2.

CAMBIO EN LA ESTRATEGIA DE ALIMENTACIÓN DE LAS HEMBRAS DEL LEÓN MARINO DEL SUR (CARNIVORA, PINNIPEDIA) TRAS EL PARTO

RESUMEN: El presente estudio pretende comprobar si las hembras del león marino sudamericano adoptan una dieta más bentónica y costera tras el parto con el fin de reducir la duración de los viajes de alimentación y así minimizar el tiempo durante el cual las crías permanecen desatendidas en la playa. Para ello, se determinaron las concentraciones de isótopos estables de carbono y nitrógeno en el suero y las células sanguíneas de 26 crías lactantes de león marino sudamericano del norte de la Patagonia, con el fin de reconstruir la dieta de la madre durante la parte final de la gestación y el inicio de la lactancia, tras la correspondiente corrección del fraccionamiento isotópico entre tejidos. También se analizaron muestras de productores primarios y de presas potenciales para ayudar en la interpretación de los resultados. Las concentraciones de isótopos estables antes y después del parto confirmaron un incremento en el consumo de presas bentónicas costeras tras el parto, apoyando así la hipótesis de un cambio generalizado de dieta tras el parto para incrementar el tiempo que pasan junto a las crías. Se discuten los efectos que esta estrategia puede tener sobre la calidad de la dieta de las hembras.

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SCIENTIA MARINA

Change in the foraging strategy of female South American sea lions (Carnivora, Pinnipedia) after parturition

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SUMMARY: This study tests the hypothesis that female South American sea lions shift from off-shore, pelagic prey to coastal, benthic prey after parturition in order to reduce the foraging trip duration and hence the time pups remain unattended on the beach during early lactation. The $\delta^{13}C$ and $\delta^{15}N$ values of the serum and blood cells of 26 South American sea lion suckling pups from northern Patagonia were used to track the dietary changes of their mothers from late pregnancy to early lactation, after correction for differential isotopic fractionation between tissues. Primary producers and potential prey species were also analysed to establish a baseline for interpreting the stable isotope concentration of serum and blood cells. Isotopic ratios revealed a generalized increase in the consumption of coastal-benthic prey after parturition. Such a generalized post-partum shift will allow females to spend more time on land and look after their pups. The effects of this foraging strategy on the nutritional quality of the female's diet are discussed.

Keywords: South American sea lion, diet, feeding strategy, suckling pup, lactating females, *Otaria flavescens*, stable isotopes, Bayesian mixing models.

INTRODUCTION

Otariids are income breeders and females alternate feeding trips to the sea with suckling bouts on land during the lactation period (Riedman, 1990). Pups remain unattended while their mothers are at sea and may die of starvation (Soto *et al*., 2004; Reid and Forcada, 2005) or because of the agonistic behaviour of other adults in the colony (Campagna *et al*., 1988; Higgins and Tedman, 1990). In this scenario, lactating otariid females are expected to modify their foraging behaviour (prey choice, time spent foraging)

in order to make foraging trip duration as short as possible while they are feeding pups more or less continuously to meet their energy requirements (Costa, 2008). These trade-offs may constrain otariid females to forage closer to the rookery during early lactation, thus promoting a dietary shift after parturition that may lead them to exploit prey not consumed throughout the year (Merrick and Loughlin, 1997; Boyd *et al*., 2002).

The South American sea lion (*Otaria flavescens*) exploits an ample range of coastal and off-shore prey in the southwestern Atlantic, foraging close to both the sea bed and the surface of the water column (Werner and Campagna, 1995; Thompson *et al*., 1998; Koen Alonso *et al*., 2000; Campagna *et al*., 2001). Stable isotope analysis has revealed that off northern Patagonia adult females consume more pelagic prey than adult males throughout the year (Drago *et al*., 2009a). However, stomach content analyses of dead females stranded primarily in summer indicate that their main prey at that time are coastal, benthic fishes and cephalopods (Koen Alonso *et al*., 2000). This apparently contradictory result could be explained if females behave for most of the year as epipelagic predators and then shift to coastal, benthic prey after parturition. Occasional observations of South American sea lion females close to the edge of the shelf during late summer suggest that their foraging ranges may expand as pups become less dependent (J.F. Mermoz, pers. comm. in Campagna *et al*., 2001), but further research is needed to clarify the situation.

Although a combination of methods is often the best approach for dietary studies, diet determination based on stomach content or scat analyses is not appropriate to test the above-mentioned hypothesis since these methods provide only a single “snapshot” of the diet of each individual just before sampling. Furthermore, repeated sampling of large animals for stomach content analysis is extremely difficult and being able to assign scats to particular individuals is highly unlikely in crowded rookeries. Conversely, stable isotope analysis offers a suitable alternative because the consumer’s tissues reflect those of its prey in a predictable manner over a long period of time (DeNiro and Epstein, 1978, 1981). The blood isotopic signal of suckling pups of pinnipeds and other mammals and that of their mothers are positively and linearly correlated, although some fractionation exists (Jenkins *et al*., 2001; Ducatez *et al*., 2008). Hence, the isotopic signal of pup tissues would reflect that of the mother’s diet, after correcting the prey-to-predator and mother-to-pup fractionations. Pups of the South American sea lions enter water for the first time when

they are about 3 or 4 weeks old (Campagna, 1985) and milk is their exclusive diet for at least the first three weeks of life, although the whole lactation period lasts approximately one year (Riedman, 1990). Furthermore, as serum and blood cells differ in isotopic turnover rate, serum half-life in endotherms being 3-4 days and that of blood cells 28-30 days (Hobson and Clark, 1993; Hilderbrand *et al.*, 1996), the isotopic signal of blood cells collected just after birth and that of serum collected three weeks later can be used respectively to infer the female diet in late pregnancy and early lactation.

In this paper, carbon and nitrogen stable isotopes of blood from suckling pups were used to reconstruct the diet of South American sea lion females during late pregnancy and early lactation and to test the hypothesis that a diet change from off-shore, pelagic prey to coastal, benthic coastal occurs in lactating South American sea lion females.

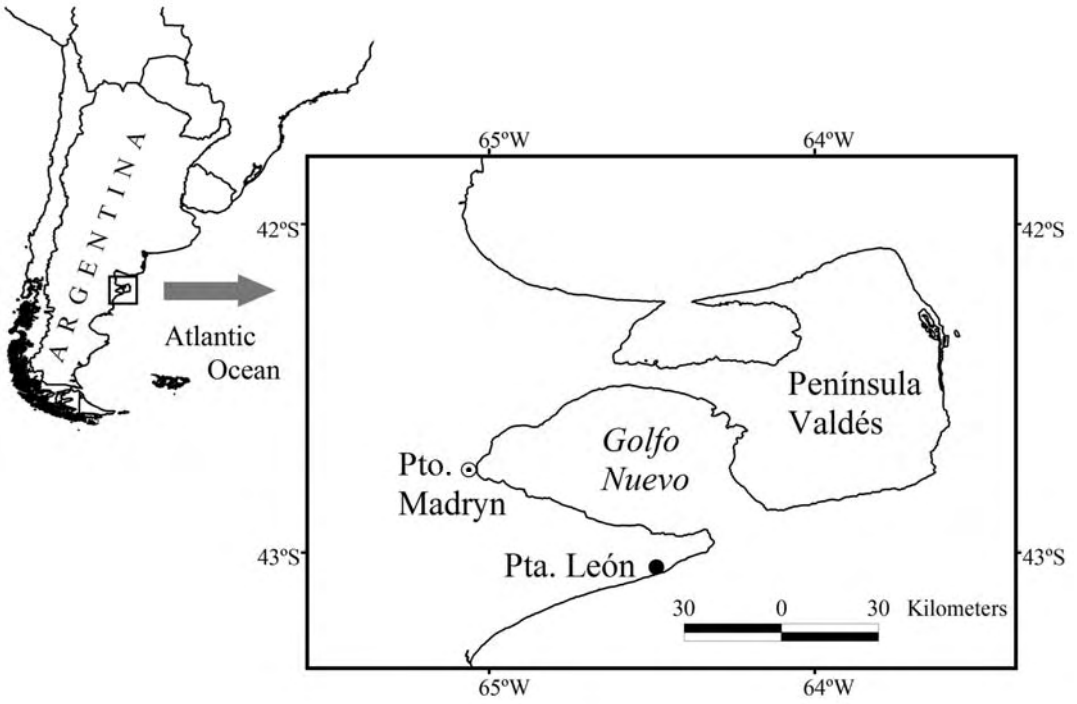

FIG. 1. - Study area.

MATERIALS AND METHODS

Study area

The present study was conducted in Punta León (43º06'S, 64º29'W), a provincial reserve, which is located on the Atlantic coast of Argentina, 25 km south of Golfo Nuevo and about 80 km from the city of Puerto Madryn, in the Chubut Province (Fig. 1). Punta León is currently one of the settlements of the species with the highest rate of annual growth and one of the most important breeding areas in northern Patagonia (Dans *et al*., 2004). During the last decade, the breeding area of Punta León has changed considerably, increasing both in number of pups and area of occupation, and new breeding areas have developed to the south of the traditional area (Dans *et al*., 2004; Grandi *et al*., 2008). The new breeding areas have a social structure different from the traditional one, as described by Campagna and Le Boeuf (1988), the proportion of juveniles of both sexes and of subadult males being higher (Grandi *et al*., 2008).

Sampling

Sampling was carried out during the 2006 breeding season, from the beginning of January to mid-February, the period when most births are registered (Campagna, 1985). Twenty-six one-week-old pups (11 males and 15 females) were captured at random using a noose pole (Gentry and Holt, 1982) and about 2-5 ml of blood was extracted from the caudal gluteal vein in the lumbar region (Geraci and Lounsbury, 1993). Pups were also sexed, bleach-marked (Campagna *et al*., 2001) and finally released close to their mothers. The entire operation took about 10-15 min for each pup. All pups were found to be readily accepted and nursed by their mother, and all of them survived to the end of the study. Two weeks after the first sampling the same pups were re-captured to extract a second blood sample.

The blood samples were centrifuged in situ at 4000 x G for 10 min without anticlotting factors to separate serum and blood cells (Cunningham, 2003). Anticlotting factors were not employed to avoid the alteration of the isotopic signal (Bosley and Wainright, 1999). Serum and blood cells were stored in liquid nitrogen and later in a freezer at -20ºC until they were used for stable isotope analysis.

Samples of nine main potential prey species for South American sea lion females off the Chubut province (Koen Alonso *et al*., 2000) were collected from the zone in January and February 2006 to determine their isotopic signals (Table 1). Off-shore pelagic potential prey species were represented by Argentine anchovy (*Engraulis anchoita*), Argentine hake (*Merluccius hubbsi*; two size classes: total length <30 cm and total length >30 cm), Argentine short-fin squid (*Illex argentinus*), South American long-fin squid (*Loligo sanpaulensis*) and Patagonian squid (*Loligo gahi*). *M. hubbsi* was classified as a pelagic species because it consumes mainly pelagic prey (Angelescu and Fuster de Plaza, 1965) and hence its assimilated nutrients are derived from the pelagic food web, although it rests on the seabed during daylight hours. Costal benthic potential prey species were represented by flounder (*Paralichthys isosceles*), banded cusk ell (*Raneya brasiliensis*), red octopus (*Enteroctopus megalocyathus*) and tehuelche octopus (*Octopus tehuelchus*). White muscle and mantle were collected from fish and cephalopods, respectively. Furthermore samples of benthic primary producers (*Codium vermilara* and *Undaria pinnatifida*) and phytoplankton (diatoms and dinoflagellates) were also collected to determine their isotopic signals and hence allow a better interpretation of isotopic results off the Chubut province. Samples of potential prey items and primary producers were provided by local fishermen or collected on board by the staff of the Laboratory of Marine Mammals of the Centro Nacional Patagónico (CENPAT). Phytoplankton was collected with a 20 μm mesh-size plankton net and, once in the laboratory, filtered in a precombusted GF/C filter. All samples were stored in a freezer at -20ºC until analysis.

Stable isotope analyses

Once in the laboratory, samples were thawed, dried in a stove at 60ºC for 36 h, and ground into a fine powder using a mortar and pestle. Lipids were extracted with a chloroform/methanol (2:1) solution (Bligh and Dyer, 1959), because lipids are depleted in ^{13}C compared with other molecules, thus confounding the results by decreasing the $\delta^{13}C$ signal (DeNiro and Epstein, 1977). As phytoplankton samples contain a high concentration of inorganic carbon that may add undesirable variability to $\delta^{13}C$ (Lorrain *et al*., 2003), they were previously treated by soaking for 24 h in 0.05 N hydrochloric acid (HCl) to decarbonize them (Ogawa and Ogura, 1997). Since HCl treatment adversely affects $\delta^{15}N$

TABLE 1. - Stable isotope values (mean ± SD) of the South American sea lion pups, the potential prey of their mothers and primary producers. Habitat type: pelagic (P) and benthic (B). Sample size (*n*).

Scientific name	Common name	Habitat	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Primary producers					
Phytoplankton		P	2[a]	-21.0 ± 0.1	13.4 ± 0.6
Seaweed					
Codium vermilara		B	5	-14.9 ± 1.3	11.6 ± 0.4
Undaria pinnatifida	wakame	B	5	-19.1 ± 1.2	10.4 ± 0.5
Potential prey					
Fish					
Engraulis anchoita	Argentine anchovy	P	5	-17.9 ± 0.2	15.7 ± 0.8
Merluccius hubbsi (>30 cm)	Argentine hake	P	5	-17.0 ± 0.5	17.1 ± 0.4
Merluccius hubbsi (<30 cm)	Argentine hake	P	5	-17.7 ± 0.6	15.9 ± 0.5
Paralichthys isosceles	Flounder	B	5	-15.9 ± 0.4	18.0 ± 0.6
Raneya brasiliensis	Banded cusk eel	B	5	-15.3 ± 0.6	18.8 ± 0.5
Cephalopods					
Illex argentinus	Argentine short-fin squid	P	5	-17.0 ± 0.6	13.7 ± 0.8
Loligo sanpaulensis	South American long-fin squid	P	5	-16.8 ± 0.2	17.2 ± 0.3
Loligo gahi	Patagonian squid	P	4	-17.6 ± 0.4	15.7 ± 0.6
Enteroctopus megalocyathus	Red octopus	B	5	-14.6 ± 0.7	18.9 ± 0.9
Octopus tehuelchus	Tehuelche octopus	B	5	-14.8 ± 0.2	19.9 ± 0.4
Sea lion pups					
Mammals					
Otaria flavescens	South American sea lion		26[b]	-15.7 ± 0.3	20.9 ± 0.6
			26[c]	-15.8 ± 0.3	23.1 ± 0.6
Benthic prey[d]			4	-15.1 ± 0.6	18.9 ± 0.8
Pelagic prey[e]			6	-17.3 ± 0.5	15.9 ± 1.3

[a], Collective samples of diatoms and dinoflagellates, including several individuals.
[b], Stable isotope value of pup blood cells from the first week.
[c], Stable isotope value of pup serum from the third week.
[d], Gross mean of the benthic prey. Sample size refers to the number of species.
[e], Gross mean of the pelagic prey. Sample size refers to the number of species.

(Bunn *et al*., 1995), each of the samples was divided into two sub-samples, one used for C analyses after decarbonation, and the other for N analyses without decarbonation.

Approximately 0.3 mg of serum, 0.25 mg of blood cells, 4.0 mg of homogenized seaweeds, 16.0 mg of homogenized phytoplankton with filter, and 0.6 mg of white muscle from fish and mantle from cephalopods were weighed into tin cups (3.3 x 5 mm), combusted at 900ºC and analyzed in a continuous flow isotope ratio mass spectrometer

(Flash 1112 IRMS Delta C Series EA; Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses. Samples were processed at Serveis Cientificotècnics de la Universitat de Barcelona.

Stable isotope abundances, expressed in delta (δ) notation, where the relative variations of stable isotope ratios are expressed in per mil (‰) deviations from predefined international standards, were calculated as:

$$\delta X = [(R_{sample} / R_{standard}) - 1]\ 10^3$$

where X is ^{13}C or ^{15}N, R_{sample} is the heavy-light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and $R_{standard}$ is the heavy-light isotope ratio in reference standards, which were the V-PDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N. International stable isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the International Atomic Energy Agency (IAEA, Vienna, Austria), namely polyethylene (IAEA CH_7, $\delta^{13}C$ = -31.8‰), graphite (IAEA $USGS_{24}$, $\delta^{13}C$ = -16.1‰), and sucrose (IAEA CH_6, $\delta^{13}C$ = -10.4‰), were used for calibration at a precision of 0.2‰. For nitrogen, international stable isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N_1, $\delta^{15}N$ = +0.4‰ and IAEA N_2, $\delta^{15}N$ = +20.3‰), and KNO_3 (IAEA NO_3, $\delta^{15}N$ = +4.7‰), were used to a precision of 0.3‰.

Data analyses

Prior to any statistical analysis, normality in data distribution was tested by Lilliefors's test. When required, homogeneity of variances was tested by Levene's contrast test. Nested ANOVA was used to compare the isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) and elemental concentration (C and N) in coastal benthic and off-shore pelagic potential prey species.

The Student's *t* test was used to investigate differences in the isotope signals ($\delta^{13}C$ and $\delta^{15}N$) in serum and blood cells of male and female pups. The same procedure was used to investigate differences between blood cell $\delta^{13}C$ and serum $\delta^{13}C$ and between blood cell $\delta^{15}N$ and serum $\delta^{15}N$ of pups, once corrected in accordance with the expected total diet-to-pup isotopic enrichment.

The Bayesian mixing model SIAR (Stable Isotope Analysis in R; Parnell *et al*., 2008) was used to calculate the relative contribution of the potential prey to the female's diet before and after parturition (i.e. in late pregnancy and early lactation). Bayesian

approaches allow incorporating not only isotopic values, elemental concentrations and fractionation factors within the mixing models, but also the uncertainties involved in all these values, thus providing results that are considerably more robust for quantifying feeding preferences than those in previous modelling approaches (Inger and Bearhop, 2008; Moore and Semmens, 2008; Parnell *et al*., 2008; Jackson *et al*., 2009). Furthermore, as the resulting later distributions of the proportions of various sources within the diet of a consumer have associated probabilities, we can use the most likely solution as a single metric for a given dietary component in subsequent analyses (Inger and Bearhop, 2008; Moore and Semmens, 2008; Jackson *et al*., 2009).

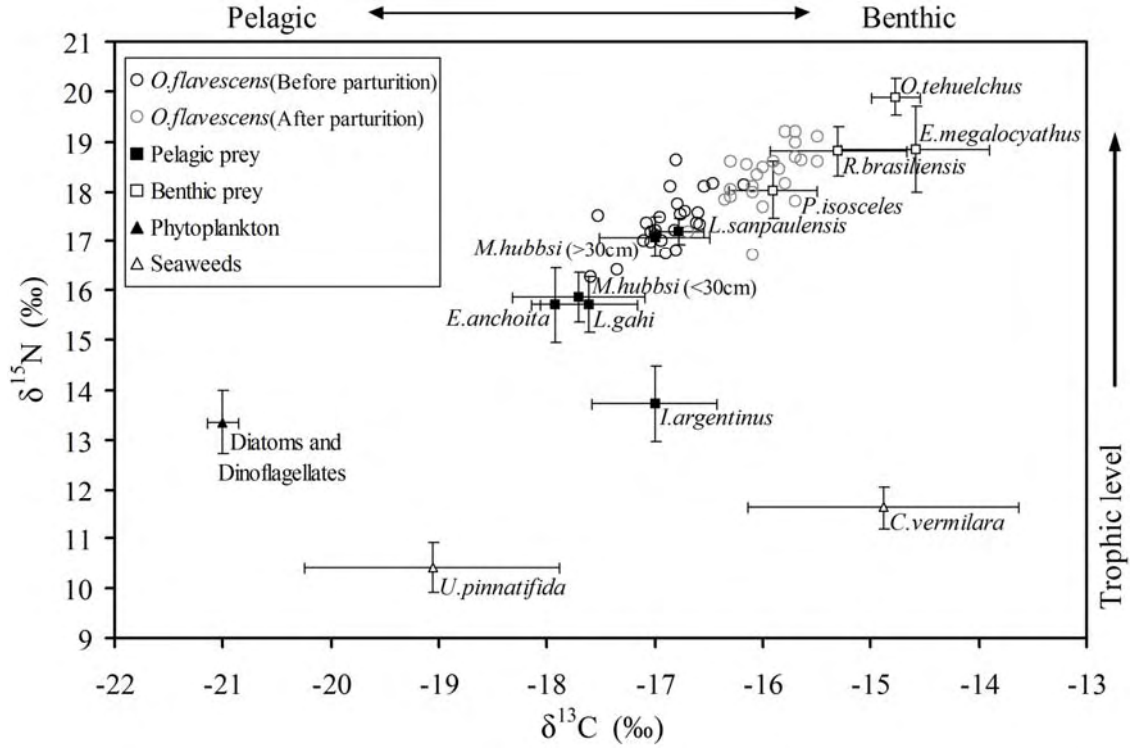

FIG. 2. - Bivariated isotopic signals of South American sea lion pups before and after parturition, once corrected in accordance with the expected total diet-to-pup isotopic enrichment for blood cells (before parturition) and serum (after parturition). Bivariated isotopic signals of the main potential prey of sea lion females and the primary producers shown as mean ± SD. Sample size n = 5 for all the species, except for *O. flavescens* (n = 26 for each tissue), *L. gahi* (n = 4) and phytoplankton (n = 2; collective samples of diatoms and dinoflagellates).

The model parameters were the isotope ratios and the elemental concentrations of the potential food sources, the isotope ratio of pup blood cells and serum and the trophic shift, or isotopic enrichment, for carbon and nitrogen from prey to predator. Prey-to-predator

isotopic enrichment in lactating female otariids has been reported to be 3.9‰ for serum $\delta^{15}N$, 0.2‰ for serum $\delta^{13}C$, 3.5‰ for blood cell $\delta^{15}N$ and 1.2‰ for blood cell $\delta^{13}C$ (Kurle, 2002). The mother-to-pup isotopic transfer is known to cause a further isotopic enrichment of 0.9‰ for serum $\delta^{15}N$, but no enrichment for serum $\delta^{13}C$ and for blood cell $\delta^{15}N$ and $\delta^{13}C$ (Jenkins *et al*., 2001). Thus, the total diet-to-pup fractionation is expected to be 4.8‰ for serum $\delta^{15}N$ and 0.2‰ for serum $\delta^{13}C$, 3.5‰ for blood cell $\delta^{15}N$ and 1.2‰ for blood cell $\delta^{13}C$. As serum and blood cells differ in the isotopic turnover rate (Hobson and Clark, 1993; Hilderbrand *et al*., 1996), blood cells from one-week-old pups were used to infer the female diet in the late pregnancy period and serum from three-week-old pups was used to infer the diet of females when feeding after parturition.

Data are usually shown as mean ± standard deviation (SD), but the feasible contribution of potential prey species to the female diet is reported as mean and the 95% credibility interval. All statistical analyses were conducted with the SPSS 15 software package, except the Bayesian mixing model, which was conducted with the SIAR software package (Parnell *et al*., 2008).

RESULTS

Table 1 shows the concentration of stable isotopes of carbon and nitrogen in the local primary producers, the potential prey, and the pups analyzed. Only the stable isotope values of blood cells from the one-week-old pups and those of serum from the three-week-old pups were analyzed. The stable isotope values of serum from the one-week-old pups and those of blood cells from the three-week-old pups were not analyzed as they are expected to be influenced both by the diet just before parturition and by the changes that might happen after parturition, thus obscuring interpretation.

The $\delta^{15}N$ of potential prey increased with trophic level and benthic primary producers were more enriched in ^{13}C than pelagic ones (Fig. 2). As a consequence, benthic potential preys were more enriched in ^{13}C and ^{15}N than pelagic ones (Table 1), as confirmed by nested ANOVA (Table 2). Differences existed among prey in elemental concentrations of carbon and nitrogen, but they were related to habitat only for carbon (Table 2).

The pup sex had no statistically significant effect on serum $\delta^{13}C$ (males: -15.9 ± 0.2‰, females: -15.7 ± 0.3‰; t = -1.243, df = 24, P = 0.226), serum $\delta^{15}N$ (males: 22.9 ± 0.6‰, females: 23.3 ± 0.6‰; t = -1.595, df = 24, P = 0.124), blood cell $\delta^{13}C$ (males: -15.6 ± 0.2‰, females: -15.7 ± 0.4‰; t = 0.545, df = 24, P = 0.591) and blood cell $\delta^{15}N$ (males: 20.8 ± 0.4‰, females: 20.9 ± 0.7‰; t = -0.536, df = 24, P = 0.597). As a consequence, male and female pups were pooled for latter analyses.

Comparison between the stable isotope values of blood cells collected when pups were one week old and those of the serum collected when pups were three weeks old, after correction for the expected diet-to-pup isotopic enrichment, revealed that corrected serum

TABLE 2. - Summary of nested ANOVA results to test for differences in the isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) and elemental concentration (C and N) of the considered prey species within habitat (benthic vs pelagic).

	SS	df	MS	*F*	*P*	r^2
$\delta^{13}C$(‰)						
Model	66.950	9	7.439	30.903	<0.001	0.877
Intersect	12659.920	1	12659.920	52592.339	<0.001	
Habitat	56.610	1	56.610	235.170	<0.001	
Species(Habitat)	10.644	8	1.331	5.527	<0.001	
Error	9.388	39	0.241			
Total	13321.060	49				
$\delta^{15}N$(‰)						
Model	155.593	9	17.288	51.296	<0.001	0.922
Intersect	14340.188	1	14340.188	42549.248	<0.001	
Habitat	107.413	1	107.413	318.709	<0.001	
Species(Habitat)	48.302	8	6.038	17.915	<0.001	
Error	13.144	39	0.337			
Total	14510.510	49				
C(%)						
Model	153.412	9	17.046	19.468	<0.001	0.818
Intersect	87047.305	1	87047.305	99417.085	<0.001	
Habitat	12.220	1	12.220	13.957	0.001	
Species(Habitat)	140.172	8	17.522	20.011	<0.001	
Error	34.148	39	0.876			
Total	89930.600	49				
N(%)						
Model	10.859	9	1.207	18.074	<0.001	0.807
Intersect	9271.528	1	9271.528	138886.01	<0.001	
Habitat	0.212	1	0.212	3.183	0.082	
Species(Habitat)	10.621	8	1.328	19.887	<0.001	
Error	2.604	39	0.067			
Total	9547.590	49				

isotopic values (δ^{13}C: -16.0 ± 0.3‰; δ^{15}N: 18.3 ± 0.6‰) were on average more enriched in both ^{13}C (t = -19.223, df = 25, P<0.001) and ^{15}N (t = -9.177, df = 25, P<0.001) than corrected blood cell isotopic values (δ^{13}C: -16.9 ± 0.3‰; δ^{15}N: 17.4 ± 0.6‰). This result is consistent with a diet including more costal, benthic prey species once females resume foraging after parturition (Fig. 2).

The results of SIAR indicated that *Merluccius hubbsi* of any size, *Engraulis anchoita* and *Loligo sanpaulensis* were the most important prey before parturition. This pattern was reversed after parturition, when females relied heavily on *Octopus tehuelchus* and other costal benthic prey (Fig. 3). As a consequence, the contribution of off-shore, pelagic potential prey to the nutrients assimilated by the females before parturition was higher than that observed after parturition (before, mean = 72%, 95% credibility interval 65-79%;

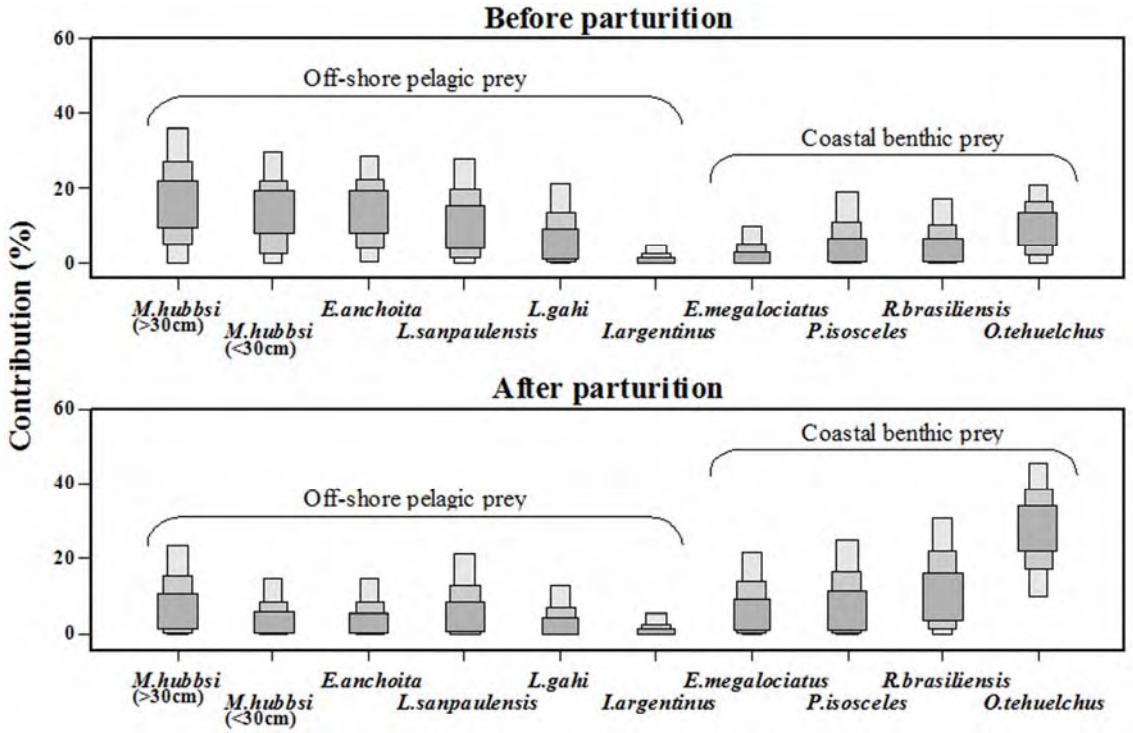

FIG. 3. - Contribution of each of the main potential prey to the female sea lion diet before (upper panels) and after parturition (lower panels), as determined by SIAR mixing model, using the isotopic signals of pup blood cells from the first week and serum from the third week respectively. Each prey species shows 95%, 75% and 50% credibility intervals for the calculated feasible contribution to the female sea lion diet.

after, mean = 32%, 95% credibility interval 25-39%), whereas the opposite was true for costal benthic potential prey (before, mean = 28%, 95% credibility interval 21-35%; after, mean = 68%, 95% credibility interval 61-75%) (Fig. 4).

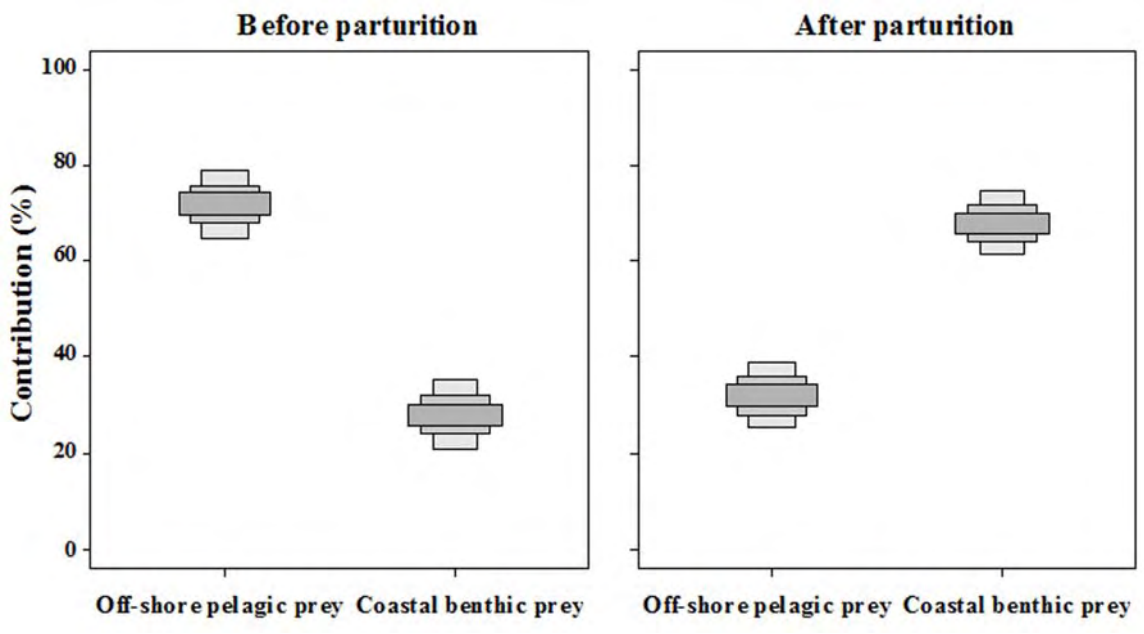

FIG. 4. - Contribution of the potential prey from off-shore, pelagic and coastal, benthic habitats to the female sea lion diet before (left panel) and after parturition (right panel), as determined by the SIAR mixing model using the isotopic signals of pup blood cells from the first week and serum from the third week, respectively. The contribution of each group of prey to the female sea lion diet is shown with 95%, 75% and 50% credibility intervals.

DISCUSSION

In recent years stable isotopes have become a standard tool for investigating trophic relationship of wild animals (Crawford *et al.*, 2008). Their use is based on three assumptions: (1) that carbon and nitrogen in the body of an animal come directly from its food and, as a consequence, the tissue isotopic signal can be used to ascertain the relative importance of feeding sources that differ in signal (DeNiro and Epstein, 1978, 1981); (2) that the assimilated organic substance gets richer in the heavy isotopes of both C and N

when passing from a trophic level to the following one, although that enrichment is greater for nitrogen than for carbon (Michener and Schell, 1994); and (3) that the change rate of the isotopic signal of each tissue depends on its metabolic rate and, hence, the time reference of dietary information is tissue-dependent (Michener and Schell, 1994).

Neither prey-to-predator and female-to-offspring enrichment rates nor the tissue turnover have been determined for the South America sea lion in controlled experiments and hence extrapolation from studies conducted on phylogenetically close organisms is required (Lewis *et al*., 2006; Hückstädt *et al*., 2007). Fortunately, the prey-to-predator enrichment rate has been determined in other pinnipeds in captivity (Hobson *et al*., 1996; Kurle, 2002), the female-to-offspring enrichment rate has been assessed in other carnivores, including pinnipeds (Jenkins *et al*., 2001; Ducatez *et al*., 2008), and the turnover rate of blood components has been established for a number of birds and mammals (Hobson and Clark, 1993; Hilderbrand *et al*., 1996). We have used the results of these studies to interpret the values found here, but caution is needed and all the results should be considered as an approximation.

The stable isotope landscape of northern Patagonia and SIAR revealed *Merluccius hubbsi* as one of the main prey of female South American sea lions in Patagonia before parturition, which coincides with the findings of stomach content analyses previously conducted on stranded individuals from the same region (Koen Alonso *et al*., 2000). This confirmation is important, because most analyses of the interaction between South American sea lions and industrial fishing in the south-western Atlantic have focused on the fishery targeting *M. hubbsi* fishery (Koen Alonso and Yodzis, 2005; Drago *et al*., 2009b). However, SIAR indicates a much higher contribution of *Engraulis anchoita* and *Loligo sanpaulensis* to the diet of female South American sea lions during late pregnancy than the stomach content analysis does (Koen Alonso *et al*., 2000). This is probably because the isotopic signal of *M. hubbsi* shorter than 30 cm is extremely close to that of *E. anchoita* and the same is true for the isotopic signal of *M. hubbsi* larger than 30 cm and that of *L. sanpaulensis*. As a consequence, SIAR attributes roughly the same relevance in the diet of female South American sea lions to the two members of each of the former groups, although *E. anchoita* and *L. sanpaulensis* are much scarcer in stomach contents than *M. hubbsi* of any size. SIAR also indicates a much higher contribution of *Octopus tehuelchus*

to the diet of female South American sea lions after parturition than the stomach content analysis does (Koen Alonso *et al*., 2000). This is perhaps due to the small size of *O. tehuelchus* compared with *Enteroctopus megalocyathus* and therefore to the difficulty of identifying the tiny beaks in stomach contents. Alternatively, this discrepancy may indicate a switch in the prey selection, possibly resulting from a change in the abundance or distribution of the two octopus species.

Whatever the reasons for those contrasting results, the differences between the corrected isotopic signals of serum and blood cells clearly reveal a marked shift after parturition towards coastal, benthic prey to the detriment of off-shore, pelagic prey, as already reported for other pinnipeds (Chilvers *et al.*, 2005). After parturition, lactating females need to satisfy the high energy requirements involved in pup rearing, particularly during the first weeks when they need to provide lipid-rich milk to their pups, so they are expected to shift to highly nutritious prey. Pelagic prey off Patagonia usually have a higher energy density than benthic prey (Eder and Lewis, 2005; Drago *et al*., 2009b,c) and hence an increase in the consumption of pelagic prey might be expected. However, the results of the present study reveal an increase in the consumption of benthic species. The energy density of *Paralichthys isosceles* is similar to that of most pelagic potential prey, but that of *Enteroctopus megalocyathus*, *Octopus tehuelchus* and *Raneya brasiliensis* is much lower (Eder and Lewis, 2005; Drago *et al*., 2009b,c). Some pinniped species rely consistently on large benthic prey because a large individual size results in rewarding meals despite a low energy density (Costa and Gales, 2003; Chilvers *et al.*, 2006). This suggests that lactating South American sea lion females might balance the average lower nutritional quality of coastal, benthic prey by targeting the largest ones. However, SIAR indicates that after parturition lactating South American sea lion females consume large amounts of small benthic species such as *O. tehuelchus* and *R. brasiliensis* instead of larger species such as *E. megalocyathus* and *P. isosceles*. The same is true in the Falkland (Malvinas) Islands, where lactating South American sea lion females consume primarily small inshore benthic notothenid fish of unknown energy density (Thompson *et al*., 1998).

Evidence supporting the hypothesis of a general decline in the nutritional quality of the diet after parturition also comes from studies on milk quality, as the fat content of the milk of female South American sea lions decreases after parturition (Werner *et al*., 1996),

whereas the opposite is true for pinnipeds, which are capital breeders (Riedman, 1990). Furthermore, the pups of the South American sea lion whose mothers maintain a high consumption of off-shore prey in their diets during early lactation grow faster than the pups whose mothers rely on benthic coastal prey (Drago *et al*., 2009c), although nothing is known about the milk quality of lactating females consuming contrasting diets. Drago *et al*. (2009c) demonstrated that differences among pups in fasting time cannot explain the above-reported results because: i) the range of $\delta^{15}N$ values in pup blood was much wider than expected accordingly with fasting duration and the documented increase in the $\delta^{15}N$ with fasting in endothermic animals, and ii) the $\delta^{13}C$ decreases with fasting in endothermic animals but it is negatively correlated with pup growth.

Thus, the overall evidence indicates that the dietary shift observed after parturition is inconsistent with a foraging strategy aiming to maximize energy intake. On the contrary, shifting to coastal, benthic prey after parturition may result in a reduction in the foraging trip duration of females, which in turn may result in a decrease in the pup mortality rate. This hypothesis is supported by the results of a telemetry study demonstrating that in northern Patagonia the trip length of lactating females was significantly correlated with the distance travelled, although females travelling off-shore swam faster than those feeding on-shore (Campagna *et al*., 2001). Furthermore, a positive correlation between the foraging trip length of lactating South American sea lion females and the pup mortality rate has also been reported off Peru (Soto *et al*., 2004, 2006). In this scenario, the foraging strategy of South American sea lion females after parturition is determined by the trade-offs between the trip length and the nutritional quality of prey. Relying only on coastal, benthic prey of poor nutritional quality will be useful to reduce the foraging trip length and hence the pup mortality during early lactation. However, this foraging strategy will negatively affect the pup growth rate and may increase the mortality rate at weaning if the weight at weaning correlates with the survival rate, as reported for other pinnipeds (McMahon *et al*., 2000).

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Capítulo 2.

Incidencia de la dieta materna y dimensión de las colonias sobre el crecimiento y la supervivencia de las crías

SECCIÓN 3.

INFLUENCIA DE LA DIETA MATERNA, REVELADA MEDIANTE ISÓTOPOS ESTABLES, SOBRE EL CRECIMIENTO DE LAS CRÍAS DEL LEÓN MARINO DEL SUR

RESUMEN: Se utilizó la señal isotópica de $\delta^{13}C$ y $\delta^{15}N$ en el suero y las células sanguíneas de 26 crías lactantes del león marino del sur en el norte de la Patagonia como indicadora de la composición de la dieta de sus madres y poder evaluar la hipótesis según la cual los hábitos de alimentación de la madre influye en el crecimiento de las crías. También se analizaron muestras de los productores primarios y de presas potenciales de las hembras para establecer los valores isotópicos de referencia y determinar la densidad de energía. La tasa específica de crecimiento de las crías se calculo tras pesarlas dos veces en un intervalo de tres semanas. La variabilidad individual en la dieta de las hembras fue grande, probablemente como consecuencia de diferencias en el comportamiento de alimentación que depende de la edad del individuo, el tamaño corporal, y / o habilidades de búsqueda de alimento. Tal como se esperaba, el crecimiento de las cría resulto influenciado por la dieta de la madre, pues las crías de las hembras que principalmente basaban su alimentación en presas pelágicas crecieron más rápidamente que las crías de las hembras cuya dieta se basaba en presas bentónicas costeras.

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Diet of lactating South American sea lions, as inferred from stable isotopes, influences pup growth

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ABSTRACT

Serum and blood cell $\delta^{13}C$ and $\delta^{15}N$ signals from 26 suckling pups of the South American sea lion from northern Patagonia were used as proxies of the composition of their mother's diet to test the hypothesis that the foraging habits of the mother influence pup growth. Samples of primary producers and the female potential prey were analyzed to establish baseline isotopic values and to determine energy density. Pups were weighed to determine specific growth rate. Individual variability in female diet was large, probably as a consequence of dissimilarities in the foraging performance which depends on the individual's age, body size and/or foraging skills. Growth of a pup was influenced by its mother's diet, as pups of females mostly relying on pelagic offshore prey were found to grow faster than those of females basing their diet on benthic coastal prey.

Key words: South American sea lion, *Otaria flavescens*, diet, growth rate, suckling pup, lactating females, stable isotopes.

INTRODUCTION

The South American sea lion, *Otaria flavescens* (Shaw 1800), is the most abundant marine mammal occurring along the southern tip of South America (Cappozzo 2002). Males and females exhibit contrasting feeding habits (Koen Alonso *et al.* 2000, Campagna

et al. 2001) due to their marked sexual dimorphism (Cappozzo 2002). In Northern Patagonia, stomach content analysis shows that the main prey in males is the offshore demersal Argentine hake, *Merluccius hubbsi*, whereas in females it is the coastal benthic red octopus, *Enteroctopus megalocyathus* (Koen Alonso *et al.* 2000).

However, time-depth-recorders and satellite tracking have revealed high levels of variability in the foraging patterns of the lactating females of South American sea lions from the same rookery (Werner and Campagna 1995, Thompson *et al.* 1998, Campagna *et al.* 2001), a phenomenon also reported for other sea lion species (Chilvers *et al.* 2005, Villegas-Amtmann *et al.* 2008). The available data indicate that some lactating females from northern Patagonia and the Falkland (Malvinas) Islands behave as benthic foragers whereas others behave as pelagic predators, in some cases even exploiting the offshore feeding grounds typically used by males (Werner and Campagna 1995, Thompson *et al.* 1998, Campagna *et al.* 2001).

Targeting coastal benthic prey might be especially convenient for lactating females as a way to reduce the intervals between suckling bouts (Trillmich 1990, Boyd *et al.* 1998, Costa 2008) and to keep females with their pups for longer periods to safeguard the pups from the risk of being killed by subadult males during the breeding season (Campagna *et al.* 1988, Werner and Campagna 1995). On the other hand, pelagic potential prey, whose highest abundance is recorded in offshore areas (Brunetti *et al.* 1998, Cousseau and Perrotta 2000), typically have a higher energy density than coastal benthic ones (Eder and Lewis 2005), although the increased travel cost may counteract the benefit of preying on offshore pelagic prey with a higher energy density (Costa 2008). Comparing the growth performance of sucking pups from females using contrasting foraging strategies is one of the available methods to investigate these trade-offs.

Although a combination of methods is often the best approach for dietary studies, diet determination based on stomach content or scat analyses is not appropriate for the present study. This is because these methods provide only a single “snapshot” of the diet of each individual just before sampling and, hence, allow the study of feeding variability among well-defined *a priori* groups, like morphotypes, sexes, or age classes, but not that of individuals, unless through repeated sampling. However, repeated sampling of large animals for stomach content analysis is extremely difficult and being able to assign scats to

particular individuals is highly unlikely in crowded rookeries. Stable isotope analysis offers a suitable alternative because the consumer tissues reflect those of their prey in a predictable manner over a long period of time (DeNiro and Epstein 1978, 1981).

The blood isotopic signal of suckling pups of pinnipeds and other mammals and that of their mothers are positively and linearly correlated, although some fractionation exists (Jenkins *et al.* 2001, Ducatez *et al.* 2008). Hence, the isotopic signal of pup tissues would reflect that of the mother's diet, after correcting the prey-to-predator and mother-to-pup fractionations. Pups of the South American sea lions enter water for the first time when they are about 3 or 4 wks old (Campagna 1985) and milk is their exclusive diet during at least their first 3 wks of life, although the whole lactation period lasts approximately one year (Oftedal *et al.* 1987). Although reconstructing precisely the diet of lactating females of the South American sea lion from the isotopic values of pups' blood is impossible because the above reported fractionation factors have not been determined experimentally for this species, pup isotopic values can be used as proxies of the mother's diet if the isotopic landscape of the foraging grounds of females is properly described. Furthermore, as serum half-life in endotherms is 3-4 d and that of blood cells is 28-30 d (Hobson and Clark 1993, Hilderbrand *et al.* 1996), the isotopic signal of blood cells collected just after birth and that of serum collected 3 wks later can be used respectively as proxies of the female diet in late pregnancy and early lactation.

In this paper, carbon and nitrogen stable isotopes of blood from suckling pups were used to characterize the diet of female South American sea lions during late pregnancy and early lactation, and to test the hypothesis that contrasting foraging strategies of nursing females affect the growth of their pups.

METHODS

Study area

This study was conducted in Punta León (43º06'S, 64º29'W), a provincial reserve, which is located on the Atlantic coast of Argentina, 25 km south of Golfo Nuevo and about 80 km from the city of Puerto Madryn, in the Chubut Province (Fig. 1). This site was chosen because one of the most important breeding areas in Northern Patagonia and has among the highest rate of annual growth (Dans *et al.* 2004). During the last decade, the

breeding area of Punta León has changed considerably, increasing both in number of pups and area of occupation, and new breeding areas have developed south of the traditional area (Dans *et al.* 2004, Grandi *et al.* 2008). These new breeding areas have a social structure different from the traditional one, as described by Campagna and Le Boeuf (1988), being higher in the proportion of juveniles of both sexes and of subadult males (Grandi *et al.* 2008).

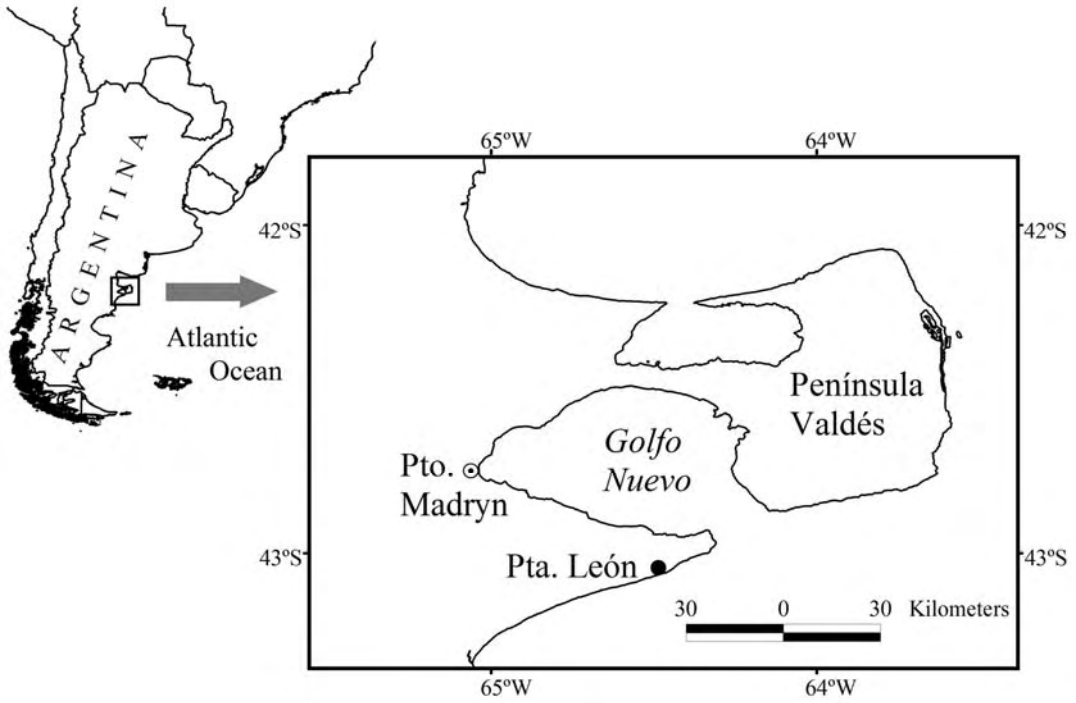

Figure 1. Study area.

Sampling

Sampling was carried out during the 2006 breeding season, extending from mid December to early February (Campagna 1985). Twenty six 1-wk-old pups (11 males and 15 females) were captured at random using a noose pole (Gentry and Holt 1982). They were placed in a nylon bag and weighed on a 50 kg (± 0.25 kg) capacity spring balance; then they were sexed, bleach-marked (Campagna *et al.* 2001), and finally released close to their mothers after extracting about 2-5 mL of blood from the caudal gluteal vein in the lumbar region (Geraci and Lounsbury 1993). The entire operation took approximately 10-

15 min for each pup. All pups were found to be readily accepted and nursed by their mother, and all of them survived to the end of the study. Two weeks later after the first sampling, the same pups were recaptured to extract a second blood sample and weighed again to calculate their specific growth rate (SGR).

The blood samples were centrifuged *in situ* at 4,000 × g for 10 min without anticlotting factors to separate serum and blood cells (Cunningham 2003). Anticlotting factors were not employed to avoid the alteration of the isotopic signal (Bosley and Wainright 1999). Serum and blood cells were stored in liquid nitrogen and, later, in a freezer at -20 ºC until they were used for stable isotope analysis.

Eight potential prey samples of female South American sea lions off Northern Patagonia, as previously identified by Koen Alonso *et al.* (2000), were collected to characterize their isotopic signal. The potential prey species were selected to balance fish and cephalopods from benthic and pelagic habitats (Table 1). Samples of seaweeds (*Codium vermilara* and *Undaria pinnatifida*) and phytoplankton (collective samples comprising diatoms and dinoflagellates), were also collected to determine their isotopic signal and hence to produce a complete and better description of the isotopic landscape off the Chubut province. The samples of the potential prey and primary producers (seaweeds and phytoplankton) were provided by local fishermen or collected by the staff of the

Table 1. Isotopic signal, energy density and proximate chemical composition (mean ± SD) of the main potential prey of the South American sea lion females analyzed in this study; (n_1) sample size for isotopic analysis, (n_2) sample size for energy density and proximate chemical composition analysis.

Scientific name	Common name	n_1	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n_2	Energy density (KJ g^{-1} wet mass)	Ash (% wet mass)	Lipid (% wet mass)	Protein (% wet mass)
Fish									
Paralichthys isosceles(B)	Flounder	5	-15.9 ± 0.4	18.0 ± 0.6	5	5.7 ± 0.8	3.7 ± 0.5	5.9 ± 1.1	13.5 ± 1.4
Raneya brasiliensis(B)	Banded cusk eel	5	-15.3 ± 0.6	18.8 ± 0.5	2	4.6 ± 0.4	3.4 ± 0.9	2.7 ± 0.3	13.5 ± 0.2
Engraulis anchoita(P)	Argentine anchovy	5	-17.9 ± 0.2	15.7 ± 0.8	5	8.4 ± 1.4	2.8 ± 0.3	12.3 ± 3.5	13.5 ± 1.2
Merluccius hubbsi(P)	Argentine hake	5	-17.7 ± 0.6	15.9 ± 0.5	5	5.1 ± 0.3	2.7 ± 0.1	5.0 ± 0.7	12.5 ± 0.5
Cephalopods									
Enteroctopus megalocyathus(B)	Red octopus	5	-14.6 ± 0.7	18.9 ± 0.9	2	4.3 ± 0.3	1.8 ± 0.3	4.2 ± 0.1	11.1 ± 1.6
Octopus tehuelchus(B)	Tehuelche octopus	5	-14.8 ± 0.2	19.9 ± 0.4	5	4.9 ± 0.4	1.4 ± 0.1	4.9 ± 0.9	12.0 ± 0.4
Illex argentinus(P)	Argentine shortfin squid	5	-17.0 ± 0.6	13.7 ± 0.8	5	5.3 ± 0.3	1.1 ± 0.3	6.5 ± 0.3	11.0 ± 1.1
Loligo gahi(P)	Patagonian squid	4	-17.6 ± 0.4	15.7 ± 0.6	4	5.6 ± 0.3	1.3 ± 0.3	6.2 ± 0.7	13.1 ± 0.4
(B)Benthic prey		4	-15.1 ± 0.6	18.9 ± 0.8	4	4.9 ± 0.6	2.6 ± 1.1	4.4 ± 1.3	12.5 ± 1.2
(P)Pelagic prey		4	-17.6 ± 0.4	15.2 ± 1.0	4	6.1 ± 1.5	2.0 ± 0.9	7.5 ± 3.3	12.5 ± 1.1

Laboratory of Marine Mammals of CENPAT. The phytoplankton was collected with a 20 μm mesh-size plankton net and, once in the laboratory, filtered in a precombusted CF/G filter and processed for isotopic determination. All potential prey and primary producer samples were stored in a freezer at -20 ºC until analysis.

Growth

The SGR, relative to the period covering from the 7th to the 21st day of age, was calculated according to the following expression:

$$SGR\ (\%) = [ln\ (W_{t+d} / W_{t0}) / d] \times 100$$

where W_{t0} is the initial body weight of the pup, W_{t+d} is the final body weight after d days and d is the number of days.

Stable Isotope Analyses

Once thawed, samples were dried at 60 ºC and ground to a fine powder with mortar and pestle. Lipids were extracted with a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) because they are depleted in ^{13}C in comparison with other molecules, thus confounding the results by decreasing the $\delta^{13}C$ signal (DeNiro and Epstein 1977). Phytoplankton was also treated with 0.05 M HCl to remove carbonates (Ogawa and Ogura 1997).

Approximately 0.3 mg of serum, 0.25 mg of blood cells, 4.0 mg of homogenized seaweeds, 16.0 mg of the homogenized phytoplankton with filter, and 0.6 mg of white muscle from fish and of mantle from cephalopods were weighed in tin cups (3.3 × 5 mm), combusted at 900 ºC and analyzed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses.

Stable isotope abundances, expressed in delta (δ) notation, in which the relative variations of stable isotope ratios are expressed in per mil (‰) deviations from predefined international standards, were calculated as:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N, R_{sample} is the heavy-light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and $R_{standard}$ is the heavy-light isotope ratio in reference standards, which were the

V-PDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and the atmospheric nitrogen (air) for ^{15}N. International isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the IAEA (International Atomic Energy Agency, Vienna, Austria), namely polyethylene (IAEA CH7, $\delta^{13}C$ = -31.8‰), graphite (IAEA USGS24, $\delta^{13}C$ = -16.1‰), and sucrose (IAEA CH6, $\delta^{13}C$ = -10.4‰), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N1, $\delta^{15}N$ = +0.4‰ and IAEA N2, $\delta^{15}N$ = +20.3‰), and KNO_3 (IAEA NO_3, $\delta^{15}N$ = +4.7‰), were used to a precision of 0.3‰.

Energy density

The proximate chemical composition (water, lipids, proteins, and ash contents) of eight potential prey species of sea lion females (Koen Alonso *et al.* 2000) was analyzed. Because carbohydrates are generally low in fish, and therefore their contribution to the energetic value is negligible (Sidwell *et al.* 1974, Craig *et al.* 1978), we did not measure their content in tissues. Once thawed, samples were weighed and dried at 100 ºC until a constant weight was reached. The moisture content was calculated by gravimetric difference between wet and dry mass (Eder and Lewis 2005). Dry samples were homogenized and a subsample burnt for 6 h in a muffle furnace at 600 ºC for ash determination (Doyle *et al.* 2007). Another subsample was processed to determine its nitrogen content by means of an elemental analyzer. This value was later multiplied by 5.8 to obtain the relative abundance of proteins in the dry material, as recommended by Gnaiger and Bitterlich (1984) for fish and other marine species. Lipids were extracted from a third subsample with a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) and their content determined by the gravimetric difference between fat and non-fat dry mass (Gnaiger and Bitterlich 1984).

To calculate the energy provided by the lipids and proteins, we used a mean combustion equivalent of these compounds of 23.9 kJ g^{-1} for proteins and 39.5 kJ g^{-1} for lipids (Clarke *et al.* 1992).

Data analyses

Prior to any statistical analysis, normality in data distribution was tested by Lilliefors's test, and their homogeneity of variances by Levene's contrast test.

Nested ANOVA was used to compare ash, lipid, and protein contents, the energy density and the isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) in coastal benthic and offshore pelagic potential prey species.

The Student's *t*-test was used to investigate differences in the SGR and serum and blood cell isotope signals ($\delta^{13}C$ and $\delta^{15}N$) of male and female pups.

Pearson's parametric correlation coefficient was used to determine whether a relation existed between blood cell $\delta^{13}C$ and serum $\delta^{13}C$ and between blood cell $\delta^{15}N$ and serum $\delta^{15}N$. The same procedure was used to determine whether a relation existed between the pup SGR and the serum isotope signal of 3-wk-old pups ($\delta^{13}C$ and $\delta^{15}N$).

Data are always shown as mean ± standard deviation (SD), unless otherwise stated. All statistical analyses were conducted with the SPSS 15 software package.

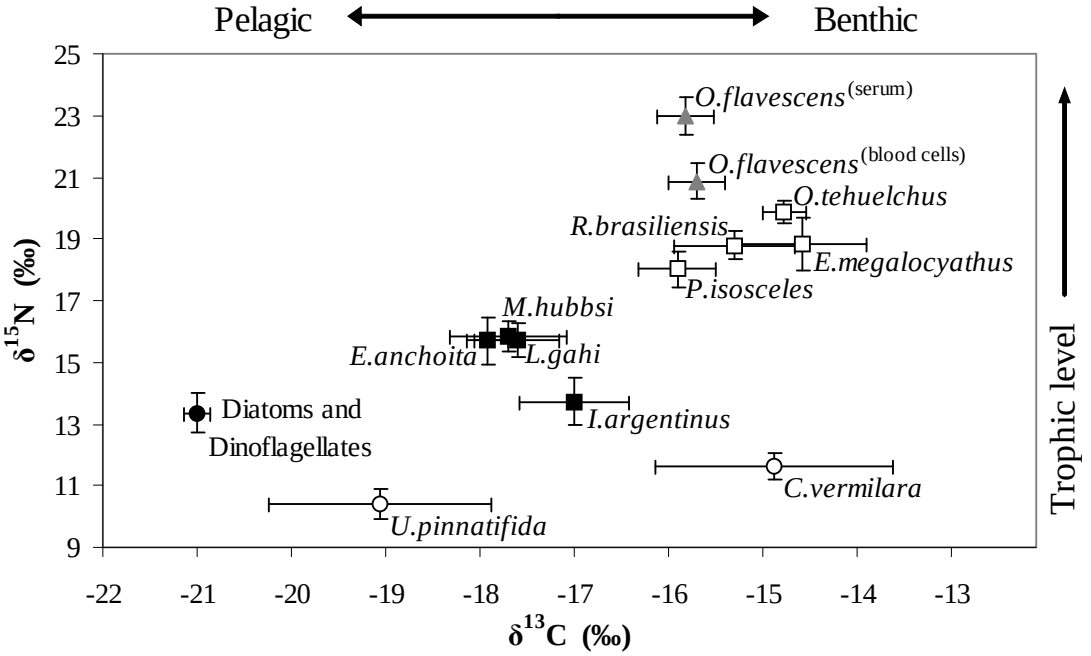

Figure 2. Bivariated isotopic signals (mean ± SD) of the South American sea lion pups, the main potential prey of their mothers and the primary producers; (▲) pups, (□) benthic prey, (■) pelagic prey, (○) seaweeds, (●) phytoplankton. Sample size: n = 5 for all the species, except for the *O. flavescens* (n = 26 for each tissue), *L. gahi* (n = 4) and phytoplankton (n = 2; collective samples of diatoms and dinoflagellates).

RESULTS

Figure 2 shows the isotopic signals of the local primary producers (phytoplankton and seaweeds) and the potential sea lion prey. The $\delta^{15}N$ increased with the trophic level, and the benthic primary producers were more enriched in ^{13}C than the pelagic ones. As a consequence, benthic potential prey were more enriched in ^{13}C, as well in ^{15}N, than pelagic ones (Table 1), as confirmed by nested ANOVA (Table 2).

Table 2. Summary of the results of nested ANOVA to test for differences in the $\delta^{13}C$ and $\delta^{15}N$ of the considered prey species within habitat (benthic *vs.* pelagic).

	SS	df	MS	*F*	*P*	r^2
$\delta^{13}C$(‰)						
Model	64.201	7	9.172	35.067	< 0.001	0.888
Intersect	10375.216	1	10375.216	39668.436	< 0.001	
Habitat	56.321	1	56.321	215.337	< 0.001	
Species(Habitat)	7.708	6	1.285	4.912	0.001	
Error	8.108	31	0.262			
Total	10463.580	39				
$\delta^{15}N$(‰)						
Model	155.544	7	22.221	56.481	< 0.001	0.927
Intersect	11295.582	1	11295.582	28711.302	< 0.001	
Habitat	129.188	1	129.188	328.372	< 0.001	
Species(Habitat)	24.007	6	4.001	10.170	< 0.001	
Error	12.196	31	0.393			
Total	11571.730	39				

Table 1 shows the energy density and the ash, protein and lipid contents of the potential prey analyzed. Nested ANOVA revealed that offshore pelagic potential prey had on average a higher energy density and lipid contents than coastal benthic ones, but lower ash contents (Table 3). Statistically significant differences were also found in the protein contents of the considered species, but on average offshore pelagic and coastal benthic potential prey did not differ in protein contents (Table 3).

Pup sex had no statistically significant effect either on SGR or on serum and blood cells $\delta^{13}C$ and $\delta^{15}N$ isotope signals (Table 4). As a consequence, male and female pups were pooled for latter analyses. Figure 2 shows the isotopic values of the blood cells from the 1-wk-old pups and those of the serum from the 3-wk-old pups. The isotopic values of the serum from the 1-wk-old pups and those of the blood cells from the 3-wk-old pups are

Table 3. Summary of the results of nested ANOVA to test for differences in the energy density and the proximate chemical composition (lipids, proteins, and ash contents) of the considered prey species within habitat (benthic *vs*. pelagic).

	SS	df	MS	*F*	*P*	r^2
Energy density						
Model	47.856	7	6.837	13.850	< 0.001	0.795
Intersect	858.217	1	858.217	1738.595	< 0.001	
Habitat	10.373	1	10.373	21.013	< 0.001	
Species(Habitat)	38.772	6	6.462	13.091	< 0.001	
Error	12.341	25	0.494			
Total	1121.564	33				
Ash						
Model	43.401	7	6.200	57.646	< 0.001	0.942
Intersect	177.867	1	177.867	1653.718	< 0.001	
Habitat	7.680	1	7.680	71.408	< 0.001	
Species(Habitat)	37.984	6	6.331	58.859	< 0.001	
Error	2.689	25	0.108			
Total	230.690	33				
Lipid						
Model	221.526	7	31.647	12.830	< 0.001	0.782
Intersect	1050.927	1	1050.927	426.051	< 0.001	
Habitat	57.669	1	57.669	23.379	< 0.001	
Species(Habitat)	166.784	6	27.797	11.269	< 0.001	
Error	61.667	25	2.467			
Total	1663.568	33				
Protein						
Model	32.089	7	4.584	4.983	0.001	0.583
Intersect	4511.675	1	4511.675	4904.215	< 0.001	
Habitat	0.152	1	0.152	0.165	0.688	
Species(Habitat)	31.679	6	5.280	5.739	0.001	
Error	22.999	25	0.920			
Total	5281.324	33				

not shown, as they are expected to be influence both by the diet just before parturition but also by the changes that might happen after parturition, thus obscuring interpretation.

Direct comparison between the isotopic values of the blood cells and the serum is not possible, as those tissues are known to differ in fractionation values (Kurle 2002). However, the average $\delta^{15}N$ of both serum and blood cells from pups was higher than that of the potential prey and the average $\delta^{13}C$ of both serum and blood cells was intermediate among that of benthic and pelagic potential prey. This is consistent with a diet including a mixture of both pelagic and benthic potential prey, although with a certain level of individual variability (Fig. 2). The existence of a positive linear correlation between blood

Table 4. Summary of the results of the Student's *t*-test to test differences in the SGR (%) and serum and blood cell isotope signals ($\delta^{13}C$ and $\delta^{15}N$; ‰) of male and female pups.

	Males (mean ± SD)	Females (mean ± SD)	*t*	df	*P*
SGR	1.1 ± 0.4	0.9 ± 0.4	1.270	24	0.216
Serum $\delta^{13}C$	-15.9 ± 0.2	-15.7 ± 0.3	-1.243	24	0.226
Serum $\delta^{15}N$	22.9 ± 0.6	23.3 ± 0.6	-1.595	24	0.124
Blood cells $\delta^{13}C$	-15.6 ± 0.2	-15.7 ± 0.4	0.545	24	0.591
Blood cells $\delta^{15}N$	20.8 ± 0.4	20.9 ± 0.7	-0.536	24	0.597

cell $\delta^{13}C$ and serum $\delta^{13}C$ (r = 0.681, P < 0.001) and between blood cell $\delta^{15}N$ and serum $\delta^{15}N$ (r = 0.587, P = 0.002) indicated that individual differences in the relative dietary importance of pelagic and benthic prey already occurred in late pregnancy (blood cell data) and were maintained in early lactation (serum data). Unfortunately, ignorance about the actual fractionation factors for the South American sea lion does not permit a precise identification of the consumed prey.

Pup SGR was negatively correlated with the serum signals of $\delta^{13}C$ (r = -0.659, P < 0.001) and $\delta^{15}N$ (r = -0.535, P = 0.005) (Fig. 3).

DISCUSSION

In recent years stable isotopes have become a standard tool for investigating trophic relationships of wild animals (Crawford *et al.* 2008). Their use is based on the assumption that carbon and nitrogen in the body of an animal come directly from its food and, as a consequence, the tissue isotopic signal can be used to ascertain the relative importance of feeding sources that differ in signal (DeNiro and Epstein 1978, 1981). Milk is the only food of suckling pups and hence the isotopic signal of pup blood is expected to reflect the diet of the mother, although modified due to prey-to-predator and female-to-offspring fractionation (Jenkins *et al.* 2001).

Prey-to-predator fractionation factors in pinniped blood cells range from 1.7‰ to 4.1‰ for the $\delta^{15}N$ and from 1.2‰ to 1.7‰ for the $\delta^{13}C$, whereas those of serum range from 3.9‰ to 5.2‰ for the $\delta^{15}N$ and from 0.2‰ to 0.6‰ for the $\delta^{13}C$, depending on the species and the nutritional status of the experimental individuals (Hobson *et al.* 1996, Kurle 2002).

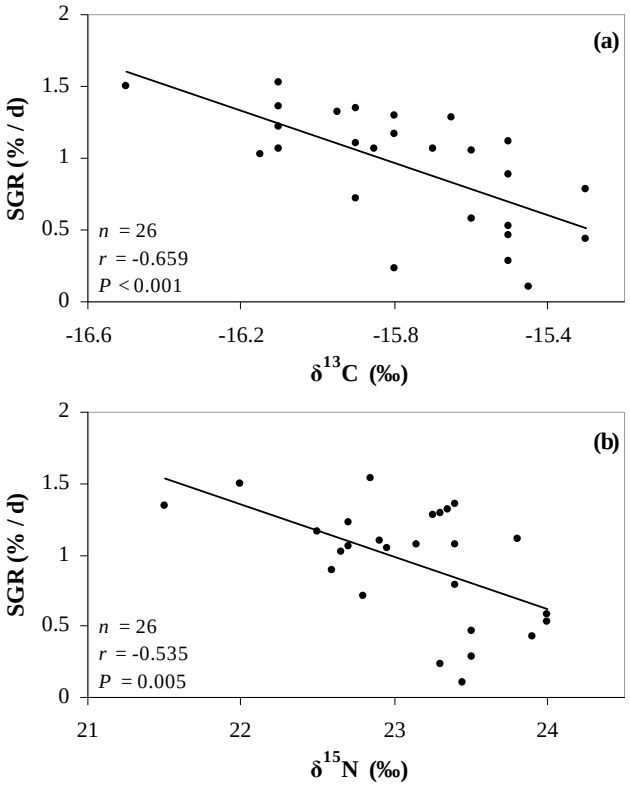

Figure 3. Correlation between carbon (a) and nitrogen (b) isotopic signal of serum from 3-wk-old pups and their SGR.

The only published female-to-offspring fractionation factors for pinnipeds refer to whole blood and are 0.3‰ for the $\delta^{13}C$, and 1.3‰ for the $\delta^{15}N$ (Ducatez *et al.* 2008). In the South American sea lion, neither the prey-to-predator and female-to-offspring enrichment rates nor the tissue turnover have been determined in controlled experiments and hence stable isotope data cannot be used to infer diet composition at the species level (but see Hückstädt *et al.* 2007). Nevertheless, individual differences in the isotopic values of pups are expected to reflect individual differences in the diet of their mothers, thus allowing comparison among individuals in the absence of other confounding factors.

Fasting is known to modify the isotopic signal of some tissues in endothermic vertebrates (Hobson *et al.* 1993, Cherel *et al.* 2005, but see Kempster *et al.* 2007). The pups of the South American sea lions fast from 1 to 10 d between suckling bouts, while females forage at sea (Campagna and Le Boeuf 1988, Campagna *et al.* 2001). However, the data published on the effects of fasting in the isotopic signal of the blood of endotherms indicate only a slight increase of the $\delta^{15}N$ of plasma (0.70‰) and a much lower increase of

the $\delta^{15}N$ of blood cells (0.24‰) after 25 d of fasting (Cherel *et al.* 2005). In this scenario, it is unlikely that a few days difference in the length of pup fasting may explain the observed variability in the $\delta^{15}N$ of pup serum (from 21.5‰ to 24.0‰).

Most importantly, Cherel *et al.* (2005) reported no effect of fasting on the $\delta^{13}C$ of blood cells and a decrease (-0.71‰) of the $\delta^{13}C$ of plasma after 25 d. If the variability of the serum $\delta^{13}C$ of the pups studied had been caused by differences in the length of the fasting period between suckling bouts, the serum $\delta^{13}C$ of the pups would be expected to decrease as the length of the fasting period increased and the growth rate decreased. However our findings indicate that the serum $\delta^{13}C$ of the pups increased as the SGR of the pups decreased. Finally, the isotopic signal of blood cells, mostly synthesized one or two months before parturition (Hobson and Clark 1993, Hilderbrand *et al.* 1996) and hence independent of the length of the fasting between suckling bouts, revealed a degree of variability similar to that of serum. This similarity confirms that the observed variability in the isotopic signal of serum is unrelated to the variability in the length of the foraging excursions of the females. Actually, the correlation existing between the isotopic signal of serum and that of blood cells indicates that females differed in their diet well before parturition and that the relative position of each female within a pelagic-benthic gradient remained unchanged after parturition.

The reason for such intrapopulation variability probably reflects dissimilarities in the individual foraging performance, which depends on age and/or body size. Pinnipeds with a larger body are physiologically more capable of exploiting benthic prey, as their skill at diving deeper is greater than those of smaller size, which results in sharp interspecific differences in foraging patterns (Costa 1991). Recently, Chilvers *et al.* (2006) and Villegas-Amtmann *et al.* (2008) also reported intraspecific differences in the diving behavior of lactating females of the New Zealand sea lion (*Phocarctos hookeri*) and Galapagos sea lion (*Zalophus wollebaeki*) caused by differences in body mass, with the lighter females feeding at shallower sites. Moreover, diving skills in pinnipeds are progressively acquired through their lifespan: dive duration, dive depth, traveling distance and swimming speed, all of them increase with age (Horning and Trillmich 1997, Bekkby and Bjørge 2000; Chilvers *et al.* 2005, 2006). Thus, it is also possible that the diet of females is strongly dependent on the age of individuals.

Whatever the reason for such variability, the data reported here indicate that pup growth was influenced by female diet. Pups of females that rely mostly on pelagic offshore prey were found to grow faster than those of females that feed on benthic coastal prey, as revealed by more positive $\delta^{13}C$ and $\delta^{15}N$ values in the latter. A similar relationship was also observed in Patagonia in Magellanic penguins, *Spheniscus magellanicus* (Forero *et al.* 2002*a*, *b*). Our results also confirm that the energy density of pelagic offshore potential prey is higher than that of potential benthic coastal prey and that the protein content in both groups is similar, thus leading to a higher nutritional value of the potential pelagic offshore prey. However, these results do not demonstrate that a higher growth rate of pups of females that feed on pelagic offshore prey is a direct consequence of a more nutritious diet, as other factors might be involved.

A faster growth rate of pups would be advantageous in this species only if it was not offset by an increase in pup mortality rate, which is likely to happen if the females are forced to abandon pups for a long period to carry out distant foraging trips (Soto *et al.* 2006). Actually, an increase in trip duration is thought to be the least preferred strategy for nursing otariids to overcome reductions in food availability (Costa 2008). Conversely, the high costs associated with benthic foraging in otariids (Costa and Gales 2003, Costa *et al.* 2004, Chilvers *et al.* 2006) and the low quality of costal benthic prey (this study) can be counterbalanced by the improved protection of pups and more frequent feeding derived from shorter foraging trips.

Unfortunately, there is no information on the differential mortality of pups as a function of coastal or offshore feeding by females in the studied rookery, but Campagna *et al.* (2001) found that, despite the fact that lactating females traveling farther away swam faster than those feeding closer to the coast, trip length was significantly correlated with the distance traveled. Furthermore, in the Peruvian population of the South American sea lion, pup mortality rate rises dramatically when reduced food availability forces females to increase their foraging trip length (Soto *et al.* 2004, 2006).

Whatever the case, the strong influence of the consumption of pelagic prey on the growth rate of pups in the Patagonian population of South American Sea lions also has bearing on its conservation. Currently, the sea lion population is about one-third of its estimated original size and it is recovering steadily (Dans *et al.* 2004). This might indicate

that it does not experience a shortage of high-quality food despite the fact that *M. hubbsi* has been heavily exploited by fishing (Koen Alonso and Yodzis 2005). This is the main pelagic species in the diet of South American Sea lions in the region (Koen Alonso *et al.* 2000). Hence, a further reduction in the abundance of this species or increased exploitation of the currently underexploited stocks of anchovy and squid (Koen Alonso and Yodzis 2005) may reduce the average growth rate of the pups and hinder the long-term recovery of the population, particularly if the goal is to reach the abundance levels recorded at the beginning of the 20th century.

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SECCIÓN 4.

INFLUENCIA DEL TAMAÑO DE LAS COLONIAS SOBRE EL BIENESTAR Y LA SUPERVIVENCIA DE LAS CRÍAS EN LOS LEONES MARINOS DEL SUR

RESUMEN: Se ha propuesto que el aumento de la tasa de mortalidad en tierra de las crías de león marino del sur nacidas en colonias pequeñas, debido a una mayor vulnerabilidad ante el acoso de los machos subadultos, podría explicar el estancamiento de la población del norte de la Patagonia tras el cese de la explotación comercial. Para comprobar esta hipótesis, se determinó la tasa de supervivencia de las crías en cinco colonias de diferente tamaño situadas en Punta León. Además, en la mayor y la menor de la cinco colonias se evaluaron la tasa de crecimiento de las crías, su estado inmunológico y la calidad de la dieta de las hembras. Los resultados indicaron que la tasa de supervivencia de las crías aumentaba con el de tamaño de la colonia y con la proporción del número de crías respecto al número de machos subadultos. Por otra parte, las crías crecían más rápidamente en la menor de las colonias, aunque la composición de la dieta de las hembras y el estatus inmunológico de las crías no se veía afectada por el tamaño de la colonia. La relación inversa entre la tasa de crecimiento de las crías y la tasa de supervivencia indicaban que la mortalidad era independiente del suministro de alimentos. Por lo tanto, y en ausencia de depredadores terrestres, el infanticidio por parte de los machos subadultos aparecía como la única fuente de mortalidad de las crías al margen de la inanición y la enfermedad. Además, la relación observada entre la tasa de supervivencia de las crías y la razón entre crías y machos subadultos se aproximaba a una curva de respuesta funcional de tipo II. En consecuencia, la causa más probable para la relación positiva observada entre el tamaño de la colonia y la tasa de supervivencia de las crías parece ser la reducción de la vulnerabilidad de las crías a los ataques de los machos subadultos, lo que apoya la hipótesis de que el estancamiento de la población después del cese de la explotación comercial fue debido a la existencia de un fenómeno de densodependencia inversa causado por el pequeño tamaño de las colonias.

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Influence of colony size on pup fitness and survival in South American sea lions

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ABSTRACT

Increased terrestrial pup mortality in small colonies due to harassment by sub-adult males has been proposed as a mechanism to explain the stagnation of South American sea lion populations after sealing ended. To test this hypothesis, pup survival rate was assessed in five northern Patagonia colonies with different sizes. Female diet quality as well as pup growth rate and immune status from the largest and smallest of these colonies were also assessed. Results indicated that the pup survival rate increased with colony size and pup-to-sub-adult male ratio. Furthermore, pups grew faster in the smallest colony, although female diet composition and pup immune status did not differ between the two colonies. Inverse relationship between pup growth rate and survival rate indicated that mortality was independent of food supply. In absence of terrestrial predators, infanticide by sub-adult males is the only mortality source other than starvation and illness and the relationship between pup survival rate and pup-to-sub-adult male ratio approached a type II functional response curve. Thus, infanticide stands as the most likely reason for the observed positive relationship between colony size and pup survival rate, supporting the hypothesis that post-sealing population stasis was caused by inverse density dependence.

Key words: South American sea lion, *Otaria byronia*, Inverse density dependency, Growth, Pup mortality, Phytohaemaglutinin skin test, Stable isotopes.

INTRODUCTION

Exploitation of marine ecosystems has resulted in a rapid depletion of top predators worldwide (Pauly *et al.* 1998, Jackson and Sala 2001, Myers and Worm 2003). The establishment of marine reserves has demonstrated that the populations of exploited

species can often recover quickly when exploitation ceases (Roberts 2004), but this is not always the case, and some marine species have failed to rebound (Myers and Worm 2005). Possible reasons for recovery failures may include the ecosystem shifting to an alternative stable state with a lower carrying capacity (Petraitis and Dudgeon 2004), the loss of critical habitat (Myers and Ottensmeyer 2004) or the existence of inverse density dependence at a low population size (Courchamp *et al.* 1999).

Many otariid species were brought to the brink of extinction during the 19th and 20th centuries due to commercial exploitation for their pelts and fat (Bonner 1982). After the harvests ended, some species quickly recovered while others failed to do so (Trites 1992, Wickens and York 1997, Gerber and Hilborn 2001, National Research Council 2002, Costa *et al.* 2006). The South American sea lion (*Otaria byronia*) was heavily exploited in the south-western Atlantic from the 1920s to the 1960s (Godoy 1963). Most of these populations had already been reduced to less than 10% of their original number when exploitation ceased (Crespo and Pedraza 1991, Reyes *et al.* 1999, Schiavini *et al.* 2004). The recovery of the sea lion population in Argentina only began in the early 1990s, after three decades of stagnation (Crespo and Pedraza 1991, Reyes *et al.* 1999, Schiavini *et al.* 2004), whereas in the nearby Falkland (Malvinas) Islands their population has yet to show signs of recovery (Thompson *et al.* 2005).

At least three independent processes have been hypothesized to explain the delay in the South American sea lions' recovery in the southwestern Atlantic after sealing ceased. Costa *et al.* (2006) noted that most otariid species that failed to recover or recovered slowly were benthic foragers that frequently operate near their physiological dive capability. As a consequence, Costa *et al.* (2006) suggested that competition with commercial fishing has hindered the recovery of benthic foragers, in sharp contrast to the rapid recovery of pelagic foragers. However, a detailed analysis of population trends (Dans *et al.* 2004) does not support this hypothesis, nor do the changes seen in the northern Patagonian sea lions' diet throughout the second half of the 20th century (Drago *et al.* 2009*a*); both the population size and the quality of the diet increased as the hake fishery developed.

Increased predation pressure on South American sea lions by killer whales (*Orcinus orca*), presumably promoted by a reduced abundance of large cetaceans from whaling, has also been proposed as an explanation for the stagnation of the South American sea lion

population since the end of sealing (Thompson *et al.* 2005, Branch and Williams 2006). Although Branch and Williams (2006) demonstrated that this is a plausible scenario, their model makes many assumptions, and supporting data on the diet and the population dynamics of killer whales are scarce.

Depensation caused by increased pup mortality has been proposed as a third mechanism to explain the stagnation of the South America sea lion post-sealing (Thompson *et al.* 2005). Starvation and infection are the two major causes of terrestrial pup mortality in several otariid species (*e.g.*, Trites 1989, Reid and Forcada 2005). Infanticide resulting from pup harassment by males, however, has been reported only for the South American sea lion and three other otariid species (Campagna *et al.* 1988*a*, 1992, Higgins and Tedman 1990, Wilkinson *et al.* 2000, Kiyota and Okamura 2005). As in other otariids, the South American sea lion exhibits a polygynous mating system; males sequester and keep females within breeding units in the central breeding area of the rookery instead of defending rigid territories (Campagna and Le Boeuf 1988). Groups of sub-adult males frequently raid the central breeding area, abduct pups and try to mount and copulate with them, which often results in pup death (Campagna *et al.* 1988*b*). As a consequence, pup mortality rate due to infanticide is much higher in breeding units outside colonies (23%) than within colonies (1.7%) (Campagna *et al.* 1992). Furthermore, harassment of females by adult males decreases as the male-to-female ratio decreases. The expected result is a lower pup survival rate in smaller colonies, which have a higher male-to-female ratio (Cassini and Fernández-Juricic 2003).

As sealing reduced both the population size and the colony size, the initial stagnation of the population post-sealing, might be caused by a lower pup survival rate if a positive relationship existed between colony size and pup survival. Unfortunately, little is known about the effect of colony size on the survival rate of pups other than that survival is higher in colonies than in isolated breeding units (Campagna *et al.* 1992). The aim of this research was to examine the relationship between colony size and pup survival rate and fitness, as revealed by growth rate and the strength of the immune response. The composition of the diet of lactating females from colonies of contrasting size was also considered, as this factor may influence pup survival rate (Drago *et al*. 2009*b*). This information will be used

to test the hypothesis that inverse density dependency exists in small colonies of the South American sea lion.

MATERIALS AND METHODS

Study area

The present study was conducted in Punta León (43º06'S, 64º29'W), located on the Atlantic coast of Argentina in the Chubut Province, 25 km south of Golfo Nuevo and about 80 km from the city of Puerto Madryn (Fig. 1). The area is characterized by 50 to 100 m high cliffs, open beaches and extensive rocky reefs (*ca*. 200 to 300 m wide). Punta León, with 1570 pups produced in 2001, is one of the most important sea lion breeding areas in northern Patagonia (Dans *et al*. 2004). Only Punta Buenos Aires (1778 pups) and Punta Norte (1975 pups), both of them in Península Valdés, produced more pups than Punta León

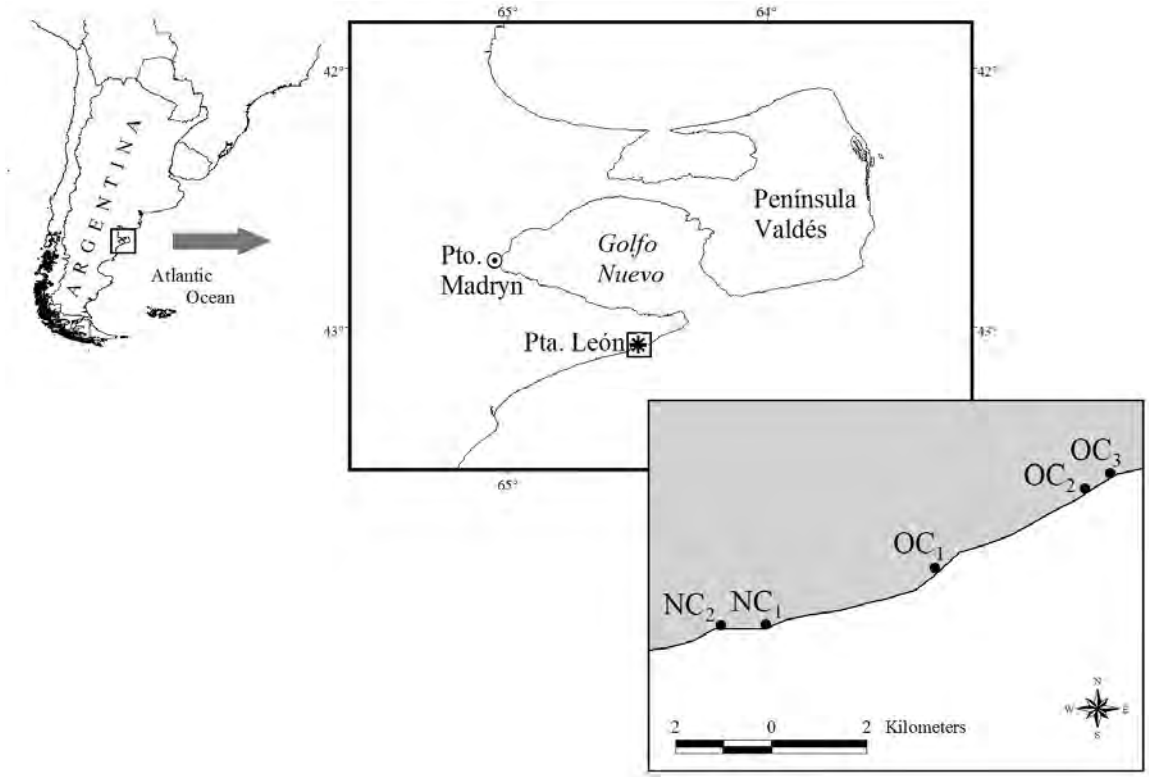

Figure 1. Study area. OC_1, OC_2, OC_3, NC_1, NC_2 show the location of the five colonies surveyed in Punta León, of which the largest is OC_1 and the smallest is NC_1.

in 2001 and the three breeding areas combined produced almost 60% of the pups born that year (Dans *et al.* 2004). Over the past ten years, the area sea lions have occupied on the beach and the number of pups have increased annually (Dans *et al.* 2004, Grandi *et al.* 2008). As a consequence, new breeding groups have settled 2 to 3 km south of the original breeding area (Dans *et al.* 2004, Grandi *et al.* 2008). These new small groups initially had a social structure different from that of typical breeding areas, as described by Campagna and Le Boeuf (1988), as they were initially formed by juveniles and included only a few females with pups, but over the years they have become true rookeries (Dans *et al.* 2004, Grandi *et al.* 2008). The entire settlement, consisting of eight colonies, covers about 10 km of coastline and the distance between the consecutive colonies ranges from 1000 to 4000 m.

Censuses

Sampling was conducted at five colonies (Fig. 1), three of them existing in the area since the time of sealing (original colonies: OC_1, OC_2, and OC_3) and two formed during the recovery process (new colonies: NC_1 and NC_2). Annual pup production at the colonies ranged from 200 to 800. All colonies were surveyed from December 20th, 2006 to February 2nd, 2007, coinciding with the peak of the breeding season (Campagna 1985). Each colony was visited, on average, weekly from December 20th to January 5th and then every three days. The numbers of adult males, sub-adult males, live pups and dead pups were counted from atop the cliff during every survey. This was possible because beaches were pebbly, without rocks that obstructed the view of animals. After each count, dead pups were removed from the beach in order to avoid recounting them during the next survey. As most dead pups lay outside the colonies, probably because they were abducted and killed by sub-adult males, the disturbance caused by the removal was very low. When dead pups lay within the colony, they were removed by a noose pole to minimize the disturbance.

Adult males and sub-adult males were distinguishable on the basis of their body shapes and colors, location in the colony and behavioral cues (Crespo and Pedraza 1991). Females (age unknown) were also counted, but their numbers were not considered for further analyses because females could not be distinguished from juveniles of either sex and the number of pups is a better indicator of the number of parturient females.

Pup sampling

Based on the previous breeding season's census, the largest (OC_1) and the smallest (NC_1) colony were selected for assessing the effect of colony size on pup fitness. Pups in the intermediate colonies were not sampled to keep disturbance as low as possible. Some pups were bleach-marked at birth in each colony and thirty of them were captured at random by a noose pole in each colony when they were two days old (Gentry and Holt 1982). Pups were placed in a holed nylon bag and weighed using a 50 kg (±0.25 kg) capacity spring balance, sexed (Cappozzo *et al.* 1991) and then released near their mothers. These procedures require more time than just marking and were not executed at birth to avoid potential interference with the mother-pup bond. The same pups were re-captured at nineteen days old, weighed for a second time to calculate their specific growth rate (SGR) (see below), subjected to the phytohaemagglutinin (PHA) skin test in order to evaluate their immune status (Smits *et al.* 1999) (see below) and had 2 to 5 mL of blood taken from the caudal gluteal vein in the lumbar region (Geraci and Lounsbury 1993). Blood stable isotope values were used as a proxy for the composition of the mother's diet (Drago *et al.* 2009*b*) and allowed testing for differences in the diet of lactating females from two colonies (OC_1 and NC_1) (see below). The entire operation took approximately 10 to 15 min for each pup. All pups were readily accepted and nursed by their mother after release.

PHA skin test

Each pup was subcutaneously injected in one flipper with 100 μg of PHA (Sigma Pharmaceuticals, Barcelona, Spain) per kg of body weight, dissolved in an equal volume of sterile phosphate buffered saline (PBS). As a control, the pup was subcutaneously injected in the other flipper with the same volume of PBS. The injection areas were bleach-marked and skin thickness was measured using a digital caliper. Each pup was re-captured 24 h after the injection in order to re-measure the thickness of the injection areas. The strength of the immune response was assessed by the difference in thickness of the flipper areas injected with PHA and PBS (Smits *et al.* 1999).

Growth

The SGR, expressed as percentage of body weight gained or lost per day (% / *d*), was calculated from the second to the nineteenth day of age according to the following equation:

$$SGR = [\ln (W_{t+d} / W_{t0}) / d] \times 100$$

where W_{t0} is the initial body weight (kg) of the pup, W_{t+d} is the final body weight (kg) after *d* days and *d* is the number of days.

Stable isotope analyses

Assuming that milk is the exclusive diet of South American sea lion pups during their first month of life and that they do not enter the water until they are about three or four weeks old (Campagna 1985), then the isotopic signal of their tissues reflects the mother's diet (Jenkins *et al.* 2001, Aurioles *et al.* 2006, Ducatez *et al.* 2008). We, therefore, used the serum from three-week-old pups as a proxy of the composition of their mothers' diet after parturition.

The blood samples were centrifuged *in situ* at 4,000 x G for 10 min to separate serum and blood cells (Cunningham 2003). Anticlotting factors were not employed in order to avoid the alteration of the isotopic signal (Bosley and Wainright 1999). Serum was placed in liquid nitrogen and later stored in a -20ºC freezer until analysis. In the laboratory, serum samples were thawed, dried at 60ºC and ground into a fine powder by mortar and pestle. Lipids were extracted with a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) because they are depleted in ^{13}C in comparison with other molecules, and thus can confound the results by decreasing the $\delta^{13}C$ signal (DeNiro and Epstein 1977, Tieszen et al. 1983).

Approximately 0.3 mg of serum were weighed in tin cups (3.3 x 5 mm), combusted at 900ºC and analyzed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses. Samples were processed at Serveis Cientificotècnics de la Universitat de Barcelona.

Stable isotope values expressed in delta (δ) notation, in which the relative variations of stable isotope ratios are expressed in per mil (‰) deviations from predefined international standards, were calculated as:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N, R_{sample} is the heavy-to-light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and $R_{standard}$ is the heavy-to-light isotope ratio in reference standards, of V-PDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and the atmospheric nitrogen (air) for ^{15}N. International isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the International Atomic Energy Agency (IAEA, Vienna), namely polyethylene (IAEA CH_7, $\delta^{13}C$ = -31.8‰), graphite (IAEA $USGS_{24}$, $\delta^{13}C$ = -16.1‰), and sucrose (IAEA CH_6, $\delta^{13}C$ = -10.4‰), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N_1, $\delta^{15}N$ = +0.4‰ and IAEA N_2, $\delta^{15}N$ = +20.3‰), and KNO_3 (IAEA NO_3, $\delta^{15}N$ = +4.7‰), were used to a precision of 0.3‰.

Data analyses

Prior to any statistical analysis, normality in data distribution was tested by a Lilliefors's test and their homogeneity of variances was tested by a Levene's contrast test.

The pup survival rate for each period was calculated by dividing the number of pups alive at the end of the period by the number of pups entering the period alive plus the number of pups born throughout the period.

The relationship between the pup survival rate and the number of pups in the colony and the relationship between the pup survival rate and the pup-to-sub-adult male ratio were investigated by logarithmic regression analysis.

A Student's *t*-test was used to investigate differences in the SGR, the immune response and the serum isotope signals ($\delta^{13}C$ and $\delta^{15}N$) of male and female pups within each of the two colonies considered (OC_1 and NC_1). The same procedure was used to test for differences in the above mentioned variables between the two colonies (OC_1 and NC_1).

A Chi-square (χ^2) test was used to detect differences in pup mortality rates between the two colonies (OC_1 and NC_1).

Data are always shown as mean ± standard deviation (SD), unless otherwise stated. All statistical analyses were conducted with the SPSS 15 software package.

RESULTS

Sub-adult males were observed in all the colonies at the beginning of the survey. Abundance of sub-adult males peaked from January 3rd to January 5th and then declined (Fig. 2). Adult males and females were observed for the first time in each of the colonies on December 28th. The number of adult males increased steadily and peaked between January 12th and January 23rd (Fig. 2). The first pup was observed in OC_1 on December 28th, but in the other four colonies pups were first observed on either January 3rd or January

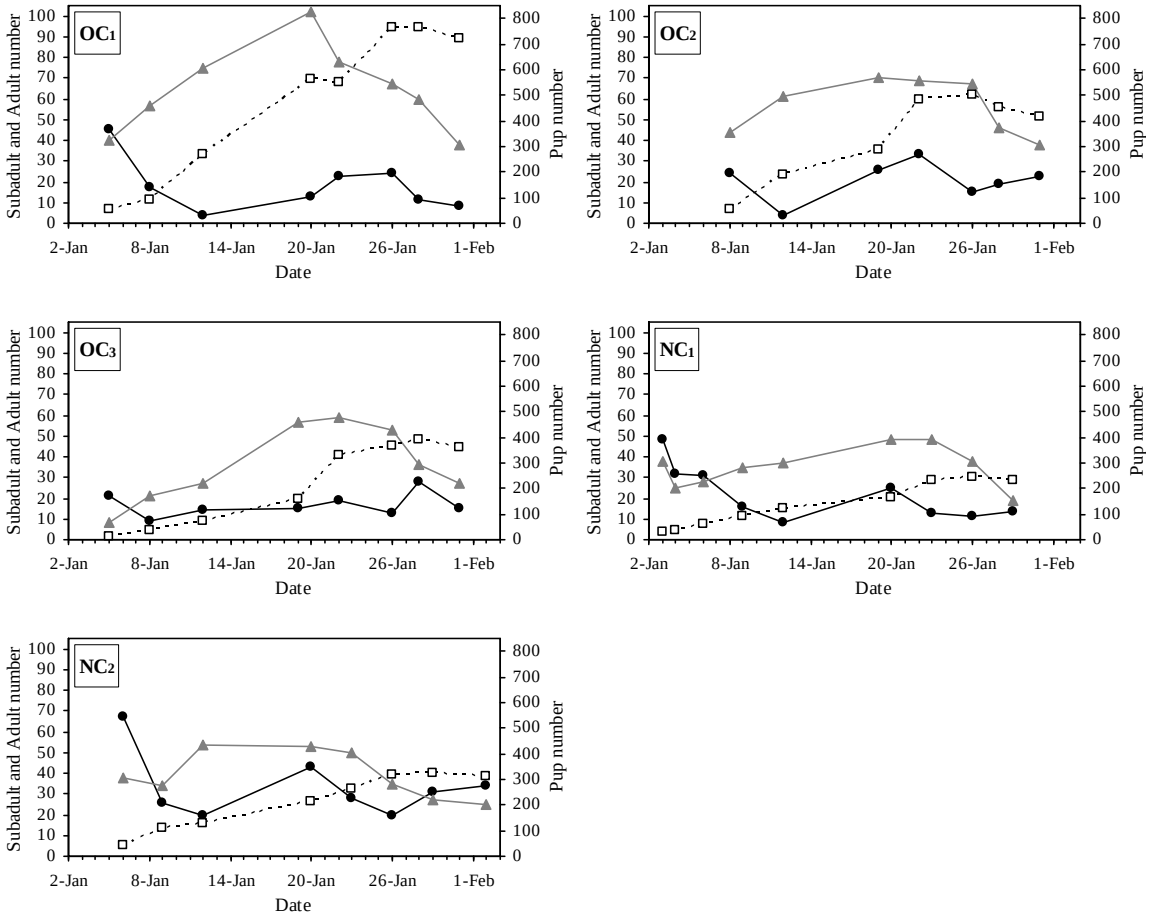

Figure 2. Number of adult males (▲), sub-adult males (●) and pups (□) in the five colonies.

5th. The number of pups increased steadily in the five colonies, peaked on either January 26th or January 28th and ranged from 231 to 721 at the end of the breeding season (Fig. 2). The pup-to-sub-adult male ratio was the lowest in the first week of January and then increased in all the colonies, not because the number of sub-adult males followed any trend, but because the number of pups increased so much that the ratio absorbed the fluctuations in the number of sub-adult males (Fig. 3). Likewise, pup survival rate was low during the first week of January and increased as the number of pups in the colony increased (Fig. 3). As a result, the number of pups in the colony and the pup-to-sub-adult male ratio were good predictors of pup survival rate, although in both cases the relationship was logarithmic and the asymptote was reached at a relatively low value of the independent variable (Fig. 4).

Initially, 60 one-week-old pups were captured from colonies OC_1 and NC_1, but only 28

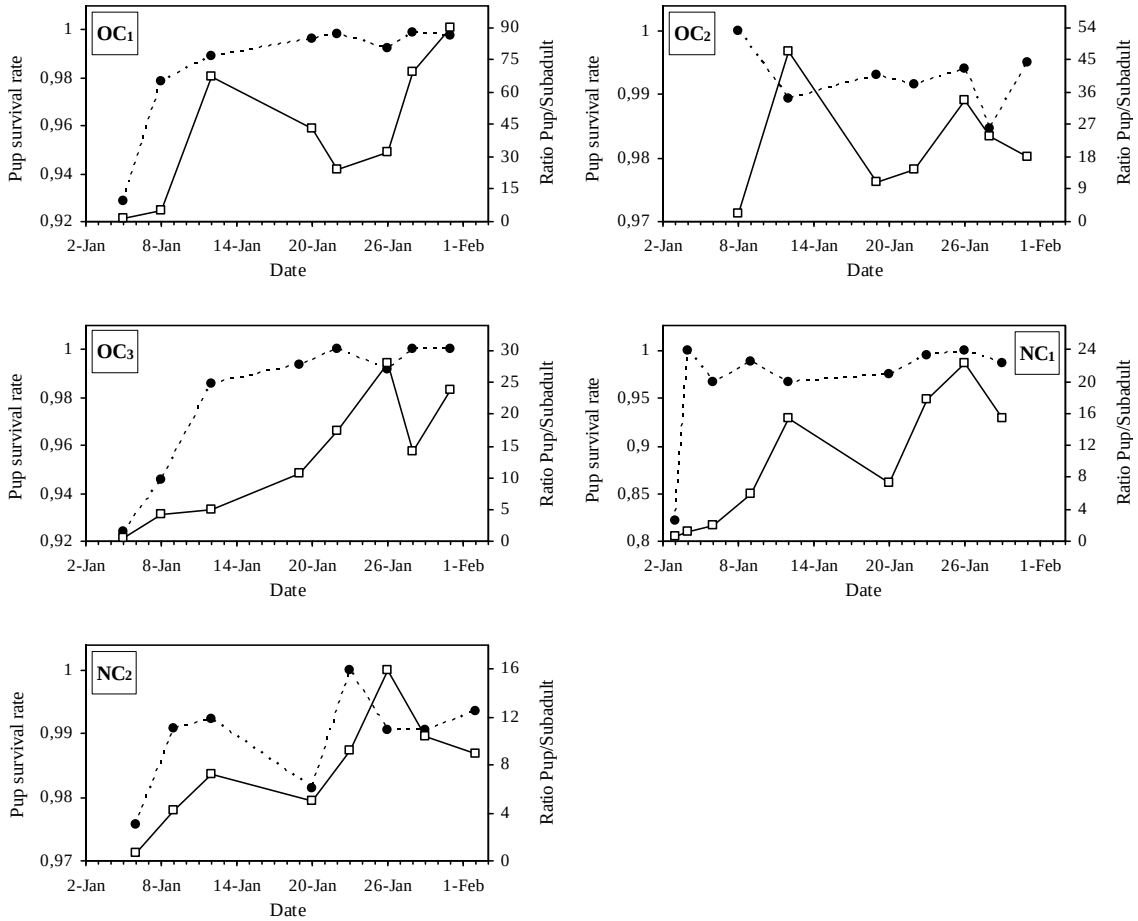

Figure 3. Temporal changes in the pup survival rate (●) and the pup-to-sub-adult male ratio (□) in the

pups were recaptured from OC_1 and 25 from NC_1. As a consequence, the stable isotope values and the SGR were computed only for those re-captured. Furthermore, the immune response was measured for only 25 pups from each of the two considered colonies because a few pups were not re-located 24 h after the first injection.

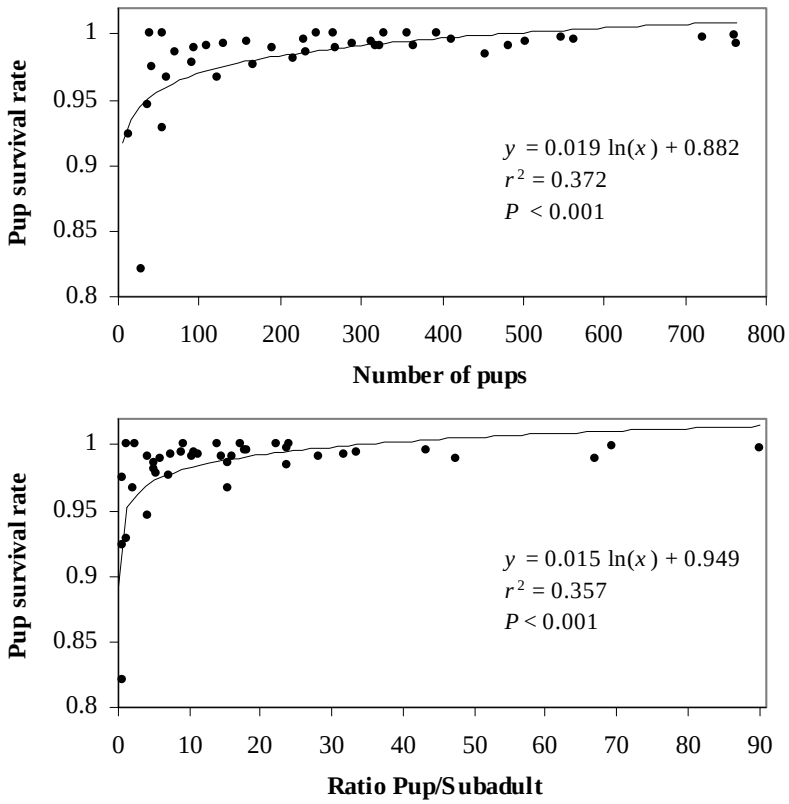

Figure 4. Effect of the number of pups in the colony and the pup-to-sub-adult male ratio on the pup survival rate in January 2007 for the five colonies considered.

Pup sex had no statistically-significant effect on SGR, serum $\delta^{13}C$, serum $\delta^{15}N$, or immune response (Table 1). As a consequence, male and female pups within each colony were pooled in later analyses. No statistically-significant differences were observed in the serum $\delta^{13}C$ (OC_1: -16.0 ± 0.4‰, NC_1: -16.1 ± 0.3‰; t = 0.842, df = 51, P = 0.404), the serum $\delta^{15}N$ (OC_1: 22.5 ± 0.6‰, NC_1: 22.3 ± 0.5‰; t = 1.000, df = 51, P = 0.322) or in the immune response (OC_1: 2.8 ± 1.6 mm, NC_1: 2.8 ± 1.7 mm; t = -0.053, df = 48, P = 0.958) of pups from the two colonies (OC_1 and NC_1). Nevertheless, the pups from the smaller (NC_1) colony grew faster than those from the larger colony (OC_1), as revealed by the

Table 1. Summary of the Student's *t*-test used to test for differences in SGR (% / *d*), serum stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; ‰) and immune response (mm) of male and female pups within each of the two colonies considered (OC_1 and NC_1).

	Males (mean ± SD)	Females (mean ± SD)	*t*	df	*P*
OC_1					
SGR	0.6 ± 0.7	0.5 ± 0.7	0.292	26	0.772
Serum $\delta^{13}C$	-15.9 ± 0.4	-16.1 ± 0.4	0.729	26	0.472
Serum $\delta^{15}N$	22.5 ± 0.7	22.4 ± 0.6	0.162	26	0.872
Immune response	3.2 ± 1.9	2.2 ± 1.0	1.632	23	0.116
NC_1					
SGR	1.4 ± 0.5	1.0 ± 0.8	1.517	23	0.143
Serum $\delta^{13}C$	-16.1 ± 0.2	-16.1 ± 0.4	-0.120	23	0.905
Serum $\delta^{15}N$	22.3 ± 0.4	22.3 ± 0.7	-0.115	23	0.910
Immune response	2.8 ± 1.3	2.8 ± 2.2	0.036	23	0.971

statistically-significant differences in mean SGR (OC_1: 0.6 ± 0.6%/*d*, NC_1: 1.3 ± 0.7%/*d*; $t = -3.740$, df = 51, $P < 0.001$). Finally, the overall pup mortality rate was lower in the larger colony (OC_1; 2.9%) than in the smaller one (NC_1; 8.7%) ($\chi^2 = 24.904$, df = 1, $P < 0.001$).

DISCUSSION

The recovery of the South American sea lion population in northern Patagonia has been a two-step process (Crespo and Pedraza 1991, Dans *et al.* 2004, Grandi *et al.* 2008). Initially, the colonies that had not been extirpated during sealing grew slowly and no new colonies were founded. The situation changed during the 1990s when the rate of pup production in the original colonies accelerated and new breeding areas were established nearby. These new colonies were initially haul-out sites, used mainly by juveniles, but over the years their social structure changed and approached that of the original colonies (Grandi *et al.* 2008). Although pup survival rate was lower in the new colonies, the annual counts of pups increased much faster in the new colonies than in the original colonies indicating that spill-over of females from the original colonies was more important than self-recruitment for the growth of the new colonies (Grandi *et al.* 2008). This expansion process is consistent with the hypothesis that intraspecific competition for resources in the

crowded, original colonies promoted the dispersal of first-time breeding females to new sites (Grandi *et al.* 2008).

Cassini and Fernández-Juricic (2003) proposed that intraspecific harassment was the main factor influencing colony selection by female South American sea lions; male harassment decreases and female-to-female interactions increase with the number of females guarded by each male. Cassini and Fernández-Juricic (2003) argued that intraspecific harassment would result in pup mortality and that females would reallocate to colonies where they experience a lower harassment to improve the chances of pup survival. However, the results presented here indicate that pup survival rate actually increases with the colony size, thus suggesting that pup mortality resulting from harassment between adults is much less important than other factors, such as infanticide by sub-adult males. Furthermore, they also indicate that lactating females may compete for food, as a larger colony size has a negative influence on pup weight gain.

Pup growth rate is known to be influenced not only by the food supply of lactating females, but also by the nutritional quality of the prey consumed (Drago *et al.* 2009*b*). Younger females are known to use more pelagic resources than older females (Drago *et al.* 2009*c*) and pelagic potential prey have a higher nutritional quality than benthic potential prey (Drago *et al.* 2009*a, b*), which results in a faster growth of those pups whose mother forage preferentially on pelagic prey (Drago *et al.* 2009*b*). As a consequence, a higher pup fitness would be expected in the smaller colonies if the females there were smaller/younger than in larger colonies. Current age structure in the studied colonies is unknown, but the absence of differences in the immune response and isotopic values of the pups from the two colonies considered indicate that the differences observed in the pups' growth rates in these colonies should be attributed to lower food availability and not to lower food quality.

Telemetry studies on South American sea lions in northern Patagonia have revealed the use of distinct foraging grounds and different movement patterns for lactating females from colonies distant *ca.* 30 km (Campagna *et al.* 2001). It might be argued that the colonies considered in the present study are only a few kilometers apart and hence the influence of food limitation on pup weight gain would operate over much larger spatial scales (Reid and Forcada 2005). However, telemetry studies have demonstrated that female northern fur seals (*Callorhinus ursinus*) from adjoining colonies, only a few kilometers

apart, also use different feeding areas, probably because slight differences in shoreline orientation result in distinct endpoints when using the same initial bearing (Robson *et al.* 2004). Another non excluding reason for the lowest growth rate of pups in the largest colony is the stress derived from more intense intraspecific harassment (Cassini and Fernández-Juricic 2003).

Regardless of the reason for why the pups' weight gain was negatively related to colony size, differences in food supply could not explain terrestrial pup mortality during the first three weeks after birth, because the pup survival rate was higher in the colony where pups grew more slowly. A lower growth rate may result in a higher pup mortality after weaning (McMahon *et al.* 2000), but this hypothesis cannot be tested with the data presented here.

Predators are known to cause inverse density dependence when the rate of predation approaches an asymptote while the abundance of prey increases (Gascoigne and Lipcius 2004). South American sea lion pups have no known predators before they enter the water (Campagna *et al.* 1992) and infanticide and starvation have been reported as the two major sources of mortality for pups younger than four weeks (Campagna *et al.* 1992). The rate of terrestrial pup survival was positively related to the number of pups in the colony and with the pup-to-sub-adult male ratio. The relationship between the pup survival rate and the pup-to-sub-adult male ratio approached a type II functional response curve, a prerequisite for predators driving inverse density dependency. Thus, harassment by sub-adult males stands as the most likely reason for a positive relationship between colony size and the rate of survival of pups younger than four weeks, as no terrestrial predators exist.

An important point to keep in mind is that the goal of sub-adult males is not to kill pups, but to copulate with females. The number of pups killed in each raid is low and highly dependent on the success of sub-adult males in seizing adult females (Campagna *et al.* 1988*a*, *b*). Under this scenario, the mortality risk for each individual pup will quickly decrease as the colony size increases, because sub-adult males are not expected to kill pups proportionally to their abundance. The number of sub-adult males in each colony fluctuates widely throughout the breeding season, but typically is high early in the season and then declines dramatically. The number of adult males in the colonies considered in the present study followed the opposite pattern, thus indicating that sub-adult males probably leave the

colonies when seizing receptive females becomes harder. As a consequence, the survival rate of pups is expected to increase. The huge increase in the number of pups during the breeding season increases even more than the pup-to-sub-adult male ratio as the breeding season goes on, leading to a higher survival rate just because of risk dilution (Krause and Ruxton 2002).

This line of reasoning supports the results presented here and those previously reported by Campagna *et al.* (1992), indicating that the vulnerability of pups to sub-adult harassment is high for isolated breeding units but quickly declines as the colony size increases and stabilizes at a colony size larger than 100 pups (present study). Interestingly, colonies with less than 100 pups were uncommon in Argentina both at the beginning of otariid exploitation (Schiavini *et al.* 2004) and again in the late 1980s when their rate of recovery accelerated (Crespo and Pedraza 1991, Reyes *et al.* 1999, Dans *et al.* 2004, Grandi *et al.* 2008). Conversely, colonies in the Falkland (Malvinas) Islands often have far fewer than 100 pups (Thompson *et al.* 2005) and hence a high mortality rate due to sub-adult male raids might be expected. This may explain why their population has not started recovering yet. The same might also be true for the Australian sea lion (*Neophoca cinerea*) and the New Zealand sea lion (*Phocarctos hookeri*). Both of these species also failed to recover immediately after seal harvests stopped (Costa *et al.* 2006), and they experience pup mortality caused by male harassment (Higgins and Tedman 1990, Wilkinson *et al.* 2000). Unfortunately, the actual relevance of infanticide in the Australian sea lion, the New Zealand sea lion and the colonies of the Southern sea lion in the Falkland (Malvinas) Islands is unknown. Research on the incidence of sub-adult male harassment in these species and populations is needed to test this hypothesis along with general studies to examine other mortality factors. More precisely, whether the relative significance of infanticide to the overall pup mortality rate declines in years when food availability is extremely low and mortality peaks due to starvation should be investigated, since the present study has covered only one breeding season, thus ignoring inter-annual variability in productivity and food supply.

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Capítulo 3.
Efecto de los cambios demográficos a largo plazo sobre la dieta y el crecimiento somático

SECCIÓN 5.

CAMBIO HISTÓRICO DE LA DIETA DEL LEÓN MARINO DEL SUR EN LA PATAGONIA REVELADO POR ANÁLISIS ISOTÓPICO

RESUMEN: Se ha utilizado el análisis isotópico de carbono y nitrógeno de tejido óseo del cráneo para investigar los efectos combinados de la caza comercial y el desarrollo de la pesca industrial sobre la dieta de león marino del sud *Otaria flavescens* en el norte de la Patagonia. También se analizaron las concentraciones de isótopos estables de carbono y nitrógeno en tejido óseo de machos de leones marinos del sur de Tierra del Fuego, donde la especie también fue diezmada por la caza pero posteriormente el desarrollo de la pesca industrial ha sido mucho menor. Los valores del $\delta^{13}C$ observados en machos y hembras del norte de la Patagonia aumentaron desde los años cuarenta hasta los años setenta, para luego disminuir de manera constante. La disminución fue similar en ambos sexos, aunque las hembras estaban más empobrecidas en ^{13}C que los machos. Los valores del $\delta^{15}N$ no cambiaron durante este tiempo en los machos, mientras que los de las hembras se redujeron desde los años cuarenta hasta la los años setenta y luego se estabilizaron. Por el contrario, no se observó ningún cambio en los valores del $\delta^{13}C$ y del $\delta^{15}N$ en los cráneos de los leones marinos de Tierra del Fuego. Como las presas bentónicas del norte de la Patagonia están más enriquecidas en ^{13}C que de las presas pelágicas, los resultados anteriores indican un aumento del consumo de presas bentónicas costeras en esta región desde los años cuarenta hasta los años setenta, cuando los leones marinos fueron diezmados por la explotación comercial, así como un aumento del consumo de presas pelágicas desde los años setenta, simultáneamente con la recuperación de la población del león marino del sur. Este cambio de dieta se puede explicar por un incremento de la competencia intraespecífica debido a la recuperación de la población de león marino y al masivo descarte de peces pelágicos por parte de los arrastreros industriales. En cambio, el escaso desarrollo de la pesca industrial frente a la Tierra del Fuego no facilito un cambio similar. No obstante, el cambio de la señal isotópica observado en el norte de la Patagonia también podría deberse, al menos parcialmente, a cambios en la productividad oceánica, si bien la evidencia en este sentido es débil.

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MARINE ECOLOGY PROGRESS SERIES

Historic diet change of the South American sea lion in Patagonia as revealed by isotopic analysis

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ABSTRACT: Carbon and nitrogen isotopic analyses of skull bone were used to investigate how sealing and the development of industrial fishing have affected the diet of the South American sea lion *Otaria flavescens* in northern Patagonia. Males from Tierra del Fuego were used as a control, as the species there was decimated by sealing, but industrial fishing is only poorly developed. The $\delta^{13}C$ of both males and females from northern Patagonia increased from the 1940s to the 1970s, and then declined steadily. The decline in the slope was similar in both sexes, although females were more depleted in ^{13}C than were males. The $\delta^{15}N$ remained unaffected in males throughout the period, whereas that of females decreased from the 1940s to the 1970s and then stabilized. Conversely, no change was found in either the $\delta^{13}C$ or $\delta^{15}N$ in the skulls from Tierra del Fuego animals. As benthic prey off northern Patagonia are more enriched in ^{13}C than pelagic prey, the above results indicate increased consumption of benthic coastal prey in this region from the 1940s to the 1970s, when sea lions were decimated by commercial hunting, and increased consumption of pelagic prey since the 1970s, simultaneous with sea lion population recovery. Reinforced intraspecific competition and massive discard of pelagic fish likely contributed to the observed dietary shift, while the poorly developed industrial fishing off Tierra del Fuego did not facilitate a similar change there. Nevertheless, physical forcing related to regime changes observed in the Pacific Ocean since the 1970s may also have played a role.

KEY WORDS: South American sea lion, *Otaria flavescens*, Dietary shift, Stable isotopes, Bone, Fishery interactions, Discarded fish

INTRODUCTION

Exploitation of marine ecosystems is causing a rapid depletion of top predators worldwide (Pauly et al. 1998, Jackson & Sala 2001, Myers & Worm 2003). As a result,

marine food webs are undergoing such extraordinary changes in their structure and function (Springer et al. 2003, Emslie & Patterson 2007) that, if the system has reached a new stable state, may prevent the restoration of the original dynamics of the ecosystem once human exploitation ceases (Petraitis & Dudgeon 2004).

Many otariid species were led to the brink of extinction during the 19th and 20th centuries, due to commercial exploitation for their pelts and fat (Bonner 1982). After killing ceased, some species quickly recovered, but others failed to do so, often because they had been out-competed by commercial fishing (Trites 1992, National Research Council 2002, Costa et al. 2006). Typically, the species that failed to recover were benthic foragers, as they often operate near their physiological dive capability and, for this reason, have a lower capacity to increase their foraging effort in response to the reduction in food availability caused by fishing (Costa et al. 2004). Fishing may not only reduce the total abundance of prey, but may also modify the structure of the ecosystem and reduce the nutritional quality of the remaining prey (Trites & Donnelly 2003). Conversely, pelagic foragers tend to recover quickly, perhaps because they benefit by the new ecosystem structure induced by human exploitation other than sealing (Hodgson & Johnston 1997, Costa et al. 2004, 2006).

In the south-western Atlantic, the South American sea lion *Otaria flavescens* was heavily exploited from the 1920s to the 1960s (Godoy 1963). Most populations had been reduced to <10% of their original number when exploitation ceased, and population recovery did not begin in Argentina until the early 1990s, after several decades of stagnation (Crespo & Pedraza 1991, Reyes et al. 1999, Schiavini et al. 2004).The population from the Falkland (Malvinas) Islands, however, has not yet recovered (Thompson et al. 2005).

The drastic reduction in the South American sea lion was not the only human impact on the ecosystem off northern Patagonia, as a high-sea fishery targeting Argentine hake *Merluccius hubbsi* was established there in the 1970s (Crespo et al. 1997, Bertolotti et al. 2001). The development of fishing dramatically reduced the hake population (Lloris et al. 2003, Koen Alonso & Yodzis 2005), which, combined with the extreme reduction if the sea lion population, led to a severe reorganization of the whole ecosystem (Koen Alonso & Yodzis 2005). Among the consequences of such changes were the increase of the

Magellanic penguin *Spheniscus magellanicus* population (Carribero et al. 1995) and several shifts in the diet of some former hake predators, like the spiny dogfish *Squalus acanthias* (Koen Alonso et al. 2002).

At first glance, these changes have not hindered the recovery of the southern sea lion population in northern Patagonia, which increased at about 6% annually from 1983 to 2002 (Dans et al. 2004). Currently, hake is a primary prey for the South American sea lion in northern Patagonia (Koen Alonso et al. 2000), and culling has been considered as a possible management strategy for sea lions in order to rebuild the hake stocks (Koen Alonso & Yodzis 2005). However, it is unknown whether South American sea lions reduced their per capita consumption of Argentine hake and increased that of other species as the hake stock declined and the population of sea lions increased, or whether they maintained a strong preference for hake, as assumed by Koen Alonso & Yodzis (2005). This issue is relevant not only to better understand the role of sea lions in the dynamics of the ecosystem, but also to determine whether alternative prey may differ in nutritional quality, a factor which could potentially influence the recovery of South American sea lions.

Inferring changes in the position of South American sea lions in the food web throughout the 20th century is one possible way to answer these questions. In the present paper, we have attempted to provide such information through retrospective analysis of stable isotopes in the skull bone of individuals stranded over the past 7 decades. The analysis of stable isotopes is a powerful means to infer food-web position changes, because the isotopic content in the tissues of a consumer reflects that of their overall prey in a predictable manner over a period of time (DeNiro & Epstein 1978, DeNiro & Epstein 1981). The extent of the time period over which this method reveals useful information depends on the biochemical turnover rate of the analysed tissue, because turnover rates of stable isotopes in tissues vary according to tissue metabolic rate. As a consequence, tissues with high turnover rates provide dietary information assimilated from recent feeding bouts, while tissues with lower turnover rates provide dietary information assimilated from more remote feeding bouts **(**Tieszen et al. 1983, Hobson & Clark 1992, Hobson 1993**)**. Bone tissue acts as a long-term integrator of isotope ratios and moderator of sporadic isotopic fluctuations, due to its relatively slow turnover, which makes it useful for comparing the

isotope ratios of many individuals over long periods of time (Schoeninger & DeNiro 1984, Lee-Thorp et al. 1989, Hirons et al. 2001).

The specific aims of the present study were: (1) to determine whether the diet of South American sea lions in northern Patagonia changed as industrial fishing and sea lion exploitation modified the structure of the whole ecosystem throughout the 20th century and (2) to assess whether any changes that occurred modified the nutritional quality of the diet.

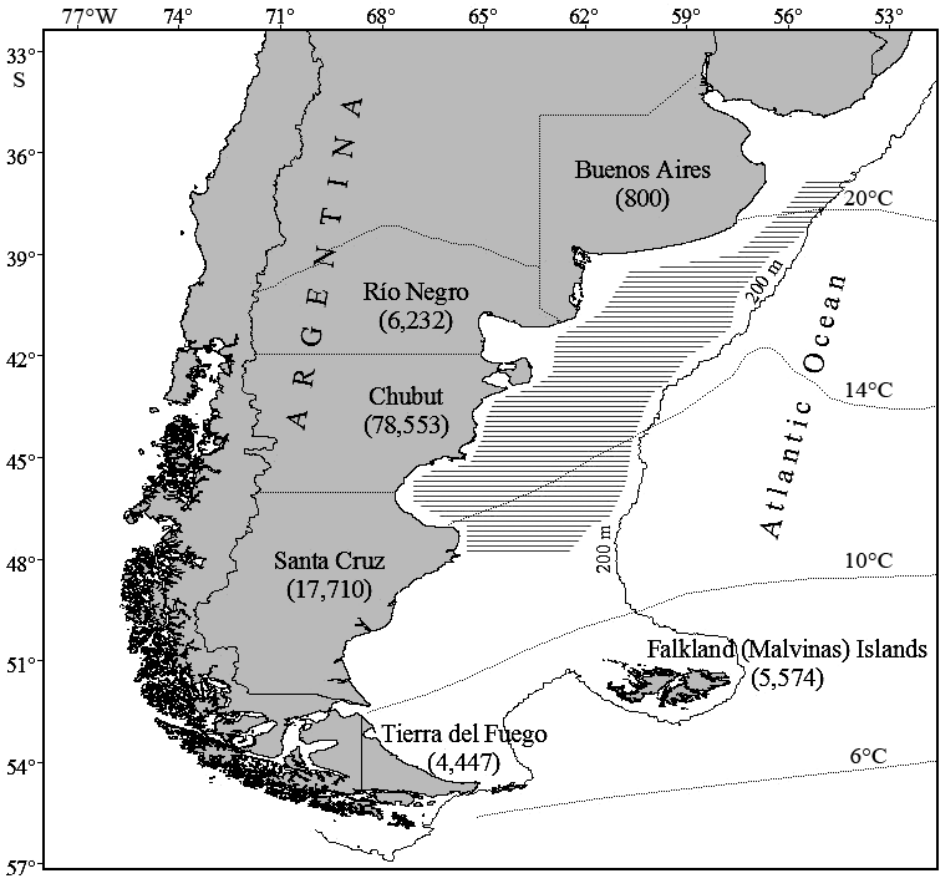

Fig. 1. *Otaria flavescens*. Distribution of the South American sea lion along the coastline of Argentina and the Falkland (Malvinas) Islands in the mid-1990s. Parentheses: population size for each province/area in agreement with Reyes et al. (1999), Dans et al. (2004), Schiavini et al. (2004), Thompson et al. (2005), Grandi et al. (2008), and G. Giardino (pers. comm.). Isotherms: sea surface temperature during summer (Hoffmann et al. 1997). Hatching: fishing grounds exploited by the Argentine industrial fishing fleet in the second half of the 1980s and in the 1990s (Giangiobbe et al. 1993, Dato et al. 2003a)

MATERIALS AND METHODS

Sampling. Fig. 1 shows the distribution of the South American sea lion *Otaria flavescens* along the coastline of Argentina and the Falkland (Malvinas) Islands and the fishing grounds exploited by the Argentine industrial fishing fleet. Skulls sampled to determine changes in the isotopic values of bone tissue came from 3 sources: (1) the collections from the Centro Nacional Patagonico (CENPAT) at Puerto Madryn, which contained skulls of individuals stranded from 1974 to 2002 along the coast of the heavily fished area off Chubut Province, in northern Patagonia (n = 30 males and n = 30 females); (2) the collection from the Acatushún Museum at Ushuaia, which contained skulls of individuals stranded from 1970 to 2007 along the coast of Tierra del Fuego, an area located about 1500 km south on the same coastline and that has been subject to much lower fishing pressure (Bertolotti et al. 2001) (n = 21 males); and (3) skulls collected for the study from a hunting site at Península Valdés (Chubut Province), which contained skeletal remains from the commercial exploitation in the 1940s, a period when the population of South American sea lions was about 60% of its original size and hake was not yet subject to industrial fishing (Fig. 2a,b) (n = 10 males and 5 females) (Bertolotti et al. 2001, Dans et al. 2003, Koen Alonso & Yodzis 2005).

All the skulls included in the present study were considered to belong to physically mature sea lions, although the age of the individuals whose skulls were sampled at the Península Valdés hunting site and the Acatushún Museum is unknown (Table 1). The individuals from the CENPAT collection had previously been aged by counting growth layer groups in the dentine of the canines (Crespo 1988, Crespo et al. 1994) and were selected according to the following criteria: a similar age (male range: 7 to 14 yr; female range: 6 to 15 yr) and adult lifetime spanning only 1 of the 3 different periods in which food availability was considered to be dissimilar. Those individuals whose adult lifetime spanned 2 of the 3 periods considered were assigned to the most recent of the periods.

The 3 periods were established in agreement with the evolution of the sea lion (Dans et al. 2003, Koen Alonso & Yodzis 2005) and hake populations (Lloris et al. 2003, Koen Alonso & Yodzis 2005) as shown in Fig. 2a,b. The first period (1965 to 1980) was characterized by high hake availability, as both the fishery landings and the population of South American sea lions were small. The second period (1981 to 1997) was characterized

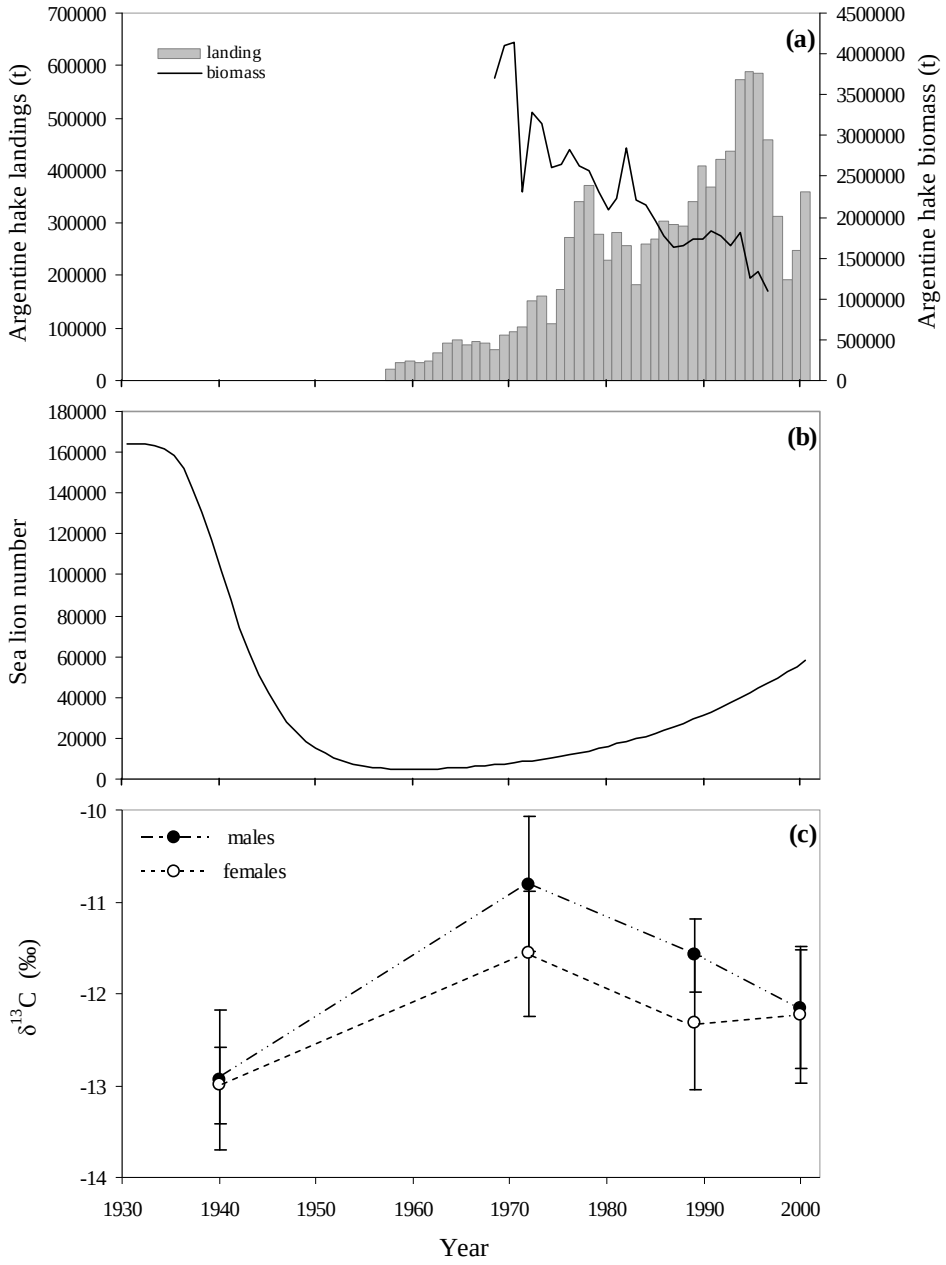

Fig. 2. (a) Hake *Merluccius hubbsi* landings (from Dans et al. 2003) and hake biomass (from Koen Alonso & Yodzis 2005), (b) trajectory of the sea lion *Otaria flavescens* population in northern and central Patagonia (from Koen Alonso & Yodzis 2005), (c) trajectory of the average $\delta^{13}C$ in the skull bone of sea lions from Chubut Province (Península Valdés hunting site and CENPAT collection) in 4 contrasting periods (present study). The year associated with each $\delta^{13}C$ value is the median of each sampling period. Error bars: SD

by declining food availability, as indicated by very large hake landings. The third period (1998 to 2002) was characterized by a population of South American sea lions in the process of recovery as well as by the collapse of the hake fishery.

Table 1. *Otaria flavescens*. Skulls of South American sea lions investigated in this study. Only 1 sample from each skull was analysed. Adult: physically mature sea lions of unknown age; 1940s: individuals from a hunting site at Península Valdés (Chubut Province) that contained skeletal remains from commercial exploitation in the 1940s

Sample number and location	Sex	Age (yr)	Dead/stranding year	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Sample number and location	Sex	Age (yr)	Dead/stranding year	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Chubut Province						OF 127	♀	6.5	1983	-12.2	22.0
1	♂	Adult	1940s	-13.4	23.1	OF 29	♀	14	1980	-10.6	22.0
2	♂	Adult	1940s	-12.1	22.2	OF 61	♀	10	1982	-10.4	24.0
3	♂	Adult	1940s	-11.9	22.9	OF 88	♀	10	1982	-11.5	21.5
4	♂	Adult	1940s	-13.3	23.9	OF 85	♀	12	1982	-11.5	21.9
5	♂	Adult	1940s	-13.7	23.7	OF 117	♀	15	1982	-11.2	21.8
6	♂	Adult	1940s	-14.0	21.8	OF 128	♀	9.5	1983	-11.9	22.2
7	♂	Adult	1940s	-12.9	21.6	OF 168	♀	9	1984	-11.5	21.8
8	♂	Adult	1940s	-11.9	23.9	OF 215	♀	8	1990	-12.3	22.2
9	♂	Adult	1940s	-13.6	22.6	OF 229	♀	11	1990	-11.9	21.8
10	♂	Adult	1940s	-12.6	24.0	OF 225	♀	12	1990	-13.1	21.6
OF 21	♂	14	1974	-11.6	21.4	OF 234	♀	13	1990	-11.7	22.2
OF 22	♂	11	1977	-10.9	24.7	OF 236	♀	7	1991	-12.3	22.1
OF 30	♂	7	1979	-10.5	23.6	OF 243	♀	7	1992	-13.0	21.9
OF 32	♂	7	1979	-11.3	23.0	OF 262	♀	9	1993	-12.0	23.3
OF 34	♂	11	1979	-11.0	21.8	OF 248	♀	10	1993	-13.6	21.6
OF 27	♂	7.3	1980	-11.6	20.7	OF 252	♀	10	1993	-11.6	22.8
OF 58	♂	8.5	1981	-9.8	22.0	OF 394	♀	8.5	1994	-12.1	22.2
OF 49	♂	8	1982	-11.5	20.2	OF 367	♀	9.5	1994	-12.0	22.6
OF 116	♂	10	1982	-9.6	22.7	OF 417	♀	10	1995	-11.8	22.4
OF 118	♂	10	1983	-10.3	22.4	OF 453	♀	13	1996	-11.5	23.4
OF 202	♂	9	1987	-12.0	21.9	OF 451	♀	12.5	1996	-11.6	22.0
OF 206	♂	12	1987	-11.0	22.4	OF 455	♀	7.5	1996	-13.3	21.7
OF 213	♂	12	1990	-11.9	23.0	OF 559	♀	10	2000	-12.6	22.0
OF 217	♂	11	1990	-11.6	22.0	OF 567	♀	15	2000	-11.1	23.2
OF 230	♂	9	1990	-11.4	19.1	OF 600	♀	6.5	2002	-13.1	21.0
OF 228	♂	12	1990	-11.0	22.4	OF 587	♀	9.5	2002	-13.0	22.4
OF 238	♂	7	1991	-11.3	24.1	**Tierra del Fuego**					
OF 245	♂	7	1992	-12.1	21.8	S/N 2	♂	Adult	1970	-12.3	23.4
OF 250	♂	9	1993	-11.9	23.2	951	♂	Adult	1981	-12.5	18.2
OF 254	♂	10	1993	-11.6	23.4	917	♂	Adult	1981	-13.2	18.5
OF 503	♂	8	1998	-12.3	22.7	1525	♂	Adult	1985	-11.6	22.4
OF 520	♂	12	1999	-12.8	21.6	1180	♂	Adult	1985	-12.9	19.7
OF 521	♂	9	1999	-11.4	22.9	1748	♂	Adult	1994	-14.9	22.2
OF 526	♂	10	2000	-12.9	20.3	2319	♂	Adult	2003	-12.7	20.2
OF 530	♂	9	2000	-12.2	22.3	2371	♂	Adult	2005	-12.5	21.3
OF 531	♂	10	2000	-12.5	21.6	2396	♂	Adult	2006	-13.4	20.9
OF 540	♂	11	2000	-12.2	20.7	2395	♂	Adult	2006	-13.8	21.0
OF 542	♂	7	2000	-11.7	21.5	2468	♂	Adult	2007	-12.9	21.3
OF 553	♂	10	2000	-12.7	20.9	2467	♂	Adult	2007	-13.7	20.6
OF 582	♂	10	2001	-10.9	23.5	2464	♂	Adult	2007	-12.9	20.5
11	♀	Adult	1940s	-12.5	24.4	2463	♂	Adult	2007	-13.2	20.6
12	♀	Adult	1940s	-13.5	24.1	2458	♂	Adult	2007	-13.6	20.5
13	♀	Adult	1940s	-13.1	23.0	2457	♂	Adult	2007	-12.0	20.9
14	♀	Adult	1940s	-13.2	23.5	2469	♂	Adult	2007	-13.5	20.7
15	♀	Adult	1940s	-12.6	23.4	2461	♂	Adult	2007	-13.0	21.2
OF 52	♀	6	1980	-11.9	21.7	2471	♂	Adult	2007	-13.1	20.6
OF 33	♀	6.1	1980	-12.6	23.1	2456	♂	Adult	2007	-12.4	21.2
OF 26	♀	13	1980	-12.0	21.6	2475	♂	Adult	2007	-13.1	20.7

The skull sample used for the isotopic analysis consisted of a small fragment of turbinate bones from the nasal cavity to avoid damaging the skull for subsequent studies (CENPAT and Acatushún Museum) or from the tympanic bulla when the turbinated bones were not in good condition (hunting site). The samples were stored dry until analysis.

Koen Alonso et al. (2000) have identified the Argentine anchovy *Engraulis anchoita*, the red octopus *Enteroctopus megalocyathus*, the Argentine short-fin squid *Illex argentinus*, the Patagonian squid *Loligo gahi*, the South American long-fin squid *Loligo sanpaulensis*, the Argentine hake *Merluccius hubbsi*, the tehuelche octopus *Octopus tehuelchus*, the flounder *Paralichthys isosceles*, and the banded cusk eel *Raneya brasiliensis* as potential prey for South American sea lions off the Chubut Province. Individuals from all these species were sampled to determine their energy density and isotopic signal. Samples of benthic primary producers (*Codium vermilara* and *Undaria pinnatifida*) and phytoplankton (diatoms and dinoflagellates) were also collected to determine their isotopic signal and, hence, to produce a complete picture of the isotopic landscape off the Chubut Province. These samples were provided by local fishermen or collected on board by staff from the CENPAT. The phytoplankton was collected with a plankton net (20 μm mesh size) and, once in laboratory, filtered with a precombusted GF/C filter. All samples were stored in a freezer at -20ºC.

Stable isotope analysis. In the laboratory, samples were thawed, when needed, dried in a stove at 60ºC for 36 h, and ground to a fine powder with a mortar and pestle. Lipids were extracted with a chloroform/methanol (2:1) solution (Bligh & Dyer 1959), because they are depleted in ^{13}C compared with other molecules and may therefore artefactually decrease the overall tissue $\delta^{13}C$ signal (DeNiro & Epstein 1977, Tieszen et al. 1983). As bone and phytoplankton samples contain high concentrations of inorganic carbon, which may cause undesirable variability to $\delta^{13}C$ (Lorrain et al. 2003), they were previously treated by soaking for 24 h in 0.5 N (bone) and 0.05 N (phytoplankton) hydrochloric acid (HCl) to decarbonise them (Ogawa & Ogura 1997, Newsome et al. 2006). Since HCl treatment adversely affects $\delta^{15}N$ (Bunn et al. 1995), each of the samples was divided into 2 subsamples: one was used for C analyses after decarbonation and the other was used for N analyses without decarbonation.

Approximately 1 mg of dried bone, 4.0 mg of homogenised seaweeds, 16.0 mg of homogenised phytoplankton with filter, and 0.6 mg of white muscle from fish and of mantle from cephalopods were weighed into tin cups (3,3 × 5 mm), combusted at 900ºC, and analysed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan). Atropine was used as a system check for elemental analyses.

Stable isotope abundances, expressed in delta (δ) notation, in which the relative variations of stable isotope ratios are expressed in per mille (‰) deviations from predefined international standards, were calculated as:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N, R_{sample} is the heavy to light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and $R_{standard}$ is the heavy to light isotope ratio of the reference standards, which were VPDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N. International isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the IAEA (International Atomic Energy Agency), namely polyethylene (IAEA CH_7, $\delta^{13}C$ = -31.8‰), graphite (IAEA $USGS_{24}$, $\delta^{13}C$ = -16.1‰) and sucrose (IAEA CH_6, $\delta^{13}C$ = -10.4‰), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N_1, $\delta^{15}N$ = +0.4‰ and IAEA N_2, $\delta^{15}N$ = +20.3‰) and KNO_3 (IAEA NO_3, $\delta^{15}N$ = +4.7‰), were used to a precision of 0.3‰.

Energy density. After thawing, samples were weighed and dried in a stove at 100ºC until a constant weight was reached. The moisture content was calculated by the gravimetric difference between wet and dry mass (Eder & Lewis 2005). The dry tissue was then homogenised, and a subsample was analysed in a mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan) to determine its nitrogen content, a value that was later multiplied by a conversion factor of 5.8 to obtain the relative richness of protein in the dry material (Gnaiger & Bitterlich 1984, Clarke et al. 1992). Another subsample was treated with a chloroform/methanol (2:1) solution to determine its lipid content by the gravimetric difference between whole tissue and fat-free tissue dry mass (Bligh & Dyer 1959).

Protein and lipid contents were converted to energy density using the mean combustion equivalents reported by Clarke et al. (1992), i.e. 23.9 and 39.5 kJ g^{-1}, respectively. Carbohydrate content was not measured, because it is generally assumed to have a negligible contribution to the energetic value of fish (Sidwell et al. 1974, Craig et at. 1978).

Data analyses. Data are always shown as mean ± standard deviation (SD), unless otherwise stated. Prior to any statistical analyses, normality of the data was tested by means of the Lilliefors test, and homoscedasticity, by means of the Levene test.

The differences in the $\delta^{13}C$, $\delta^{15}N$ and energy density of the prey species from the 2 considered habitats (benthic *vs.* pelagic) were tested by means of nested ANOVA.

The differences in the bone isotopic signal of males and females from the different sampling periods in Chubut Province (Península Valdés hunting site and CENPAT collection) were tested by 2-way ANOVA (sex × period). One-way ANOVA, followed by a Tukey (when the sample size was homogeneous among sampling periods) or Scheffé (when the sample size was not homogenous among sampling periods) post hoc test was carried out to compare the isotopic signal of individuals of the same sex in the 4 periods considered.

Time trends in the isotopic signal of the bones from the CENPAT and the Acatushún Museum collections were investigated by linear regression analysis, although the non-parametric Spearman's ρ correlation coefficient was used when data did not fit the criteria for normality. The resulting equations were compared by means of coincidence and parallelism tests (Zar 1984): the first of these assesses the hypothesis that the 2 linear regression equations are coincidental because they do not differ either in their slope or elevation and the second test assesses the hypothesis that the 2 linear regression equations do not differ in their slope.

Because the sample sizes of skulls from the CENPAT and the Acatushún Museum were different (30 skulls from the CENPAT *vs.* 21 skulls from the Acatushún Museum) and the distributions of the years when individuals stranded were not identical, we performed a randomization analysis to investigate whether such differences might influence the sensibility of the regressions fitted to the data. Thus, 100 randomized

populations with the same sample size and stranding year distribution as those from the Acatushún Museum were created from the CENPAT dataset and then a linear regression between the isotopic signal and the stranding year was computed for each randomized population. The possibility of detecting a significant correlation was not considered to be hindered by the structure of the datasets if at least 95% of the randomized populations exhibited a positive correlation between year of stranding and isotopic signal.

All statistical analyses were conducted with the SPSS 15 software package.

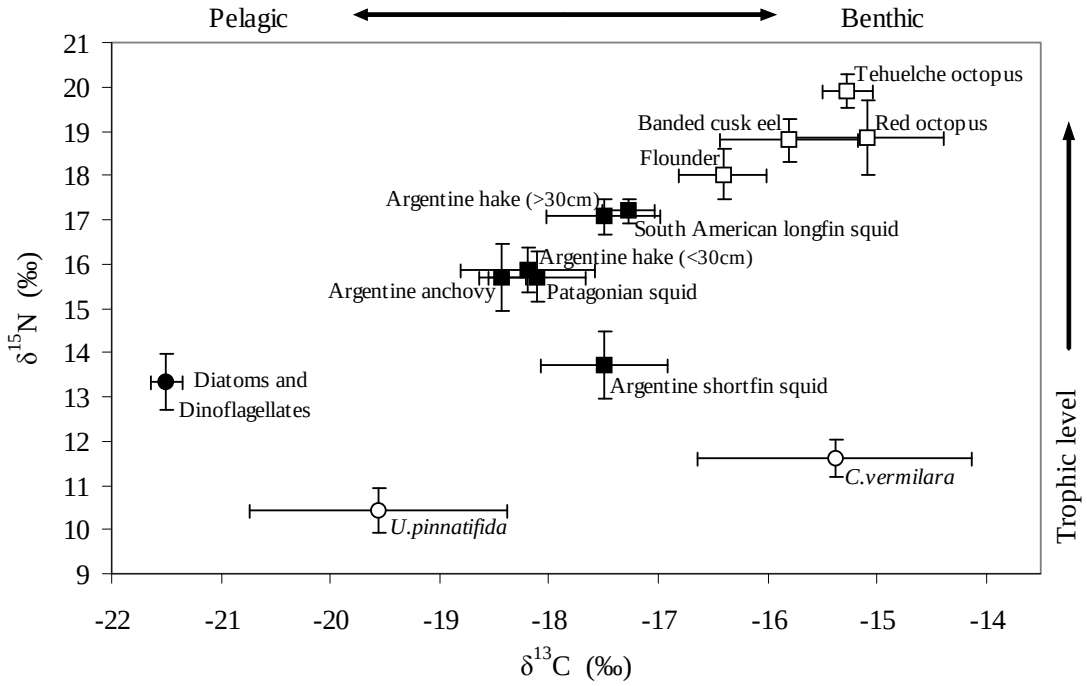

Fig. 3. Bivariated isotopic signal of the primary producers and main prey of the South American sea lion *Otaria flavescens* off the Chubut Province. Primary producers: seaweeds (○), phytoplankton (●). Main prey: benthic (□), pelagic (■). Error bars: SD

RESULTS

The $\delta^{13}C$ of the primary producers ranged from -14.9 ± 1.3‰ for seaweeds to -21 ± 0.1‰ for phytoplankton. That of the potential prey of South American sea lions *Otaria flavescens* ranged from -14.6 ± 0.7‰ for benthic species like the red octopus *Enteroctopus megalocyathus* to -17.9 ± 0.2‰ for pelagic prey like the Argentine anchovy *Engraulis anchoita* (Fig. 3, Table 2). Although adult hakes *Merluccius hubbsi* are often considered to

be demersal pelagic, their $\delta^{13}C$ is closer to that of pelagic species than to that of benthic species (Fig. 3, Table 2). This is because adult hakes spend daylight hours close to the sea bed, but move to the top layers of the water column at night to feed on pelagic species (Angelescu & Fuster de Plaza 1965). For this reason, in latter analyses, adult hakes have been included as members of the pelagic assemblage.

Table 2. *Otaria flavescens*. Mean (±SD) isotopic signals of primary producers and of the potential prey of the South American sea lion, and energy densities of potential sea lion prey, off Chubut Province (northern Patagonia, Argentina). P: pelagic habitat; B: benthic habitat; n_1: sample size for isotope analysis; n_2: sample size for energy density; (-) no data

Scientific name	Common name	Habitat	Sampling location	Sampling date	n_1	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n_2	Energy density (KJ g^{-1} wet mass)
Primary producers									
Phytoplankton		P	42º50'S, 64º42'W	Jan 2006	2[a]	-21.0 ± 0.1	13.4 ± 0.6	–	—
Seaweed									
Codium vermilara		B	42º47'S, 65º06'W	Jan 2006	5	-14.9 ± 1.3	11.6 ± 0.4	–	—
Undaria pinnatifida	wakame	B	42º47'S, 65º06'W	Jan 2006	5	-19.1 ± 1.2	10.4 ± 0.5	–	—
Potential Prey									
Fish									
Merluccius hubbsi (> 30 cm)	Argentine hake	P	47º10'S, 64º39'W	Feb 2006	5	-17.0 ± 0.5	17.1 ± 0.4	–	—
Merluccius hubbsi (< 30 cm)	Argentine hake	P	47º10'S, 64º39'W	Feb 2006	5	-17.7 ± 0.6	15.9 ± 0.5	5	5.1 ± 0.3
Engraulis anchoita	Argentine anchovy	P	42º59'S, 62º27'W	Jan 2006	5	-17.9 ± 0.2	15.7 ± 0.8	5	8.4 ± 1.4
Paralichthys isosceles	Flounder	B	42º59'S, 62º27'W	Jan 2006	5	-15.9 ± 0.4	18.0 ± 0.6	5	5.7 ± 0.8
Raneya brasiliensis	Banded cusk eel	B	42º58'S, 61º57'W	Jan 2006	5	-15.3 ± 0.6	18.8 ± 0.5	2	4.6 ± 0.4
Cephalopod									
Illex argentinus	Argentine shortfin squid	P	47º10'S, 64º39'W	Feb 2006	5	-17.0 ± 0.6	13.7 ± 0.8	5	5.3 ± 0.3
Loligo sanpaulensis	South American longfin squid	P	42º59'S, 62º27'W	Jan 2006	5	-16.8 ± 0.2	17.2 ± 0.3	5	6.2 ± 0.6
Loligo gahi	Patagonian squid	P	42º59'S, 62º27'W	Jan 2006	4	-17.6 ± 0.4	15.7 ± 0.6	4	5.6 ± 0.3
Enteroctopus megalocyathus	Red octopus	B	42º39'S, 64º59'W	Feb 2006	5	-14.6 ± 0.7	18.9 ± 0.9	2	4.3 ± 0.3
Octopus tehuelchus	Tehuelche octopus	B	43º06'S, 64º19'W	Feb 2006	5	-14.8 ± 0.2	19.9 ± 0.4	5	4.9 ± 0.4
Benthic prey[b]					4	-15.1 ± 0.6	18.9 ± 0.8	4	4.9 ± 0.6
Pelagic prey[c]					6	-17.3 ± 0.5	15.9 ± 1.3	5	6.1 ± 1.3

[a]Collective samples of diatoms and dinoflagellates including several individuals; [b]Gross mean of benthic prey. Sample size refers to the number of species; [c]Gross mean of pelagic prey. Sample size refers to the number of species

When benthic and pelagic prey were compared, the nested ANOVA (Table 3) showed that benthic prey ($\delta^{13}C$: -15.1 ± 0.6‰; $\delta^{15}N$: 18.9 ± 0.8‰) were more enriched both in ^{13}C and ^{15}N than the pelagic prey ($\delta^{13}C$: -17.3 ± 0.5‰; $\delta^{15}N$: 15.9 ± 1.3‰). The nested ANOVA also indicated that benthic prey had a lower average energy density than did

pelagic prey, although the energy density also differed among species from the same habitat (Tables 2 & 3). The isotopic signal and energy density of the red octopus and hake, the 2 main prey species of South American sea lions (Koen Alonso et al. 2000), showed

Table 3. Nested ANOVA to test for differences in the $\delta^{13}C$, $\delta^{15}N$ and energy density of the considered prey species within habitat (benthic *vs.* pelagic)

	SS	df	MS	*F*	p	r^2
$\delta^{13}C$(‰)						
Model	66.950	9	7.439	30.903	< 0.001	0.877
Intersect	12659.920	1	12659.920	52592.339	< 0.001	
Habitat	56.610	1	56.610	235.170	< 0.001	
Species(Habitat)	10.644	8	1.331	5.527	< 0.001	
Error	9.388	39	0.241			
Total	13321.060	49				
$\delta^{15}N$(‰)						
Model	155.593	9	17.288	51.296	< 0.001	0.922
Intersect	14340.188	1	14340.188	42549.248	< 0.001	
Habitat	107.413	1	107.413	318.709	< 0.001	
Species(Habitat)	48.302	8	6.038	17.915	< 0.001	
Error	13.144	39	0.337			
Total	14510.510	49				
Energy density						
Model	49.233	8	6.154	13.061	< 0.001	0.783
Intersect	954.248	1	954.248	2025.230	< 0.001	
Habitat	11.824	1	11.824	25.095	< 0.001	
Species(Habitat)	38.781	7	5.540	11.758	< 0.001	
Error	13.664	29	0.471			
Total	1317.425	38				

Table 4: *Otaria flavescens*. Two-way ANOVA tests (sex × period) performed to compare the $\delta^{13}C$ and the $\delta^{15}N$ of both sexes of South American sea lions in the 4 periods considered

	SS	df	MS	*F*	p	r^2
$\delta^{13}C$(‰)						
Model	32.917	7	4.702	10.438	< 0.001	0.522
Intersect	10359.815	1	10359.815	22994.355	< 0.001	
Period	25.996	3	8.665	19.234	< 0.001	
Sex	2.774	1	2.774	6.157	0.016	
Period*Sex	2.227	3	0.742	1.648	0.187	
Error	30.186	67	0.451			
Total	10879.910	75				
$\delta^{15}N$(‰)						
Model	16.590	7	2.370	2.505	0.024	0.207
Intersect	35852.152	1	35852.152	37887.323	< 0.001	
Period	15.144	3	5.048	5.334	0.002	
Sex	0.961	1	0.961	1.016	0.317	
Period*Sex	2.450	3	0.817	0.863	0.465	
Error	63.401	67	0.946			
Total	37617.970	75				

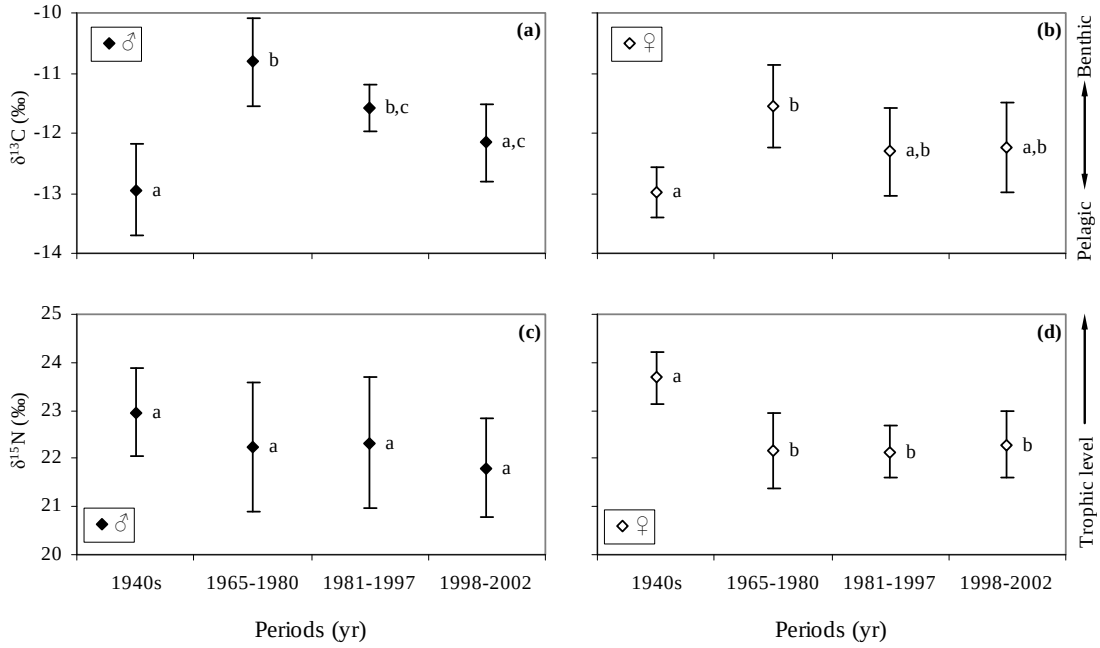

Fig. 4. *Otaria flavescens*. Mean (±SD) values of $\delta^{13}C$ (a, b) and $\delta^{15}N$ (c, d) for bone from the skulls of South American sea lions collected in Chubut Province (Península Valdés hunting site and CENPAT collection) in 4 contrasting periods. Periods with different lower-case letters differ in their mean values. Sample size: n = 10 for each sex and period, except females in the 1940s (n = 5)

the same pattern, as the red octopus was more enriched in ^{13}C and ^{15}N and had a lower energy density than hake (Fig. 3, Table 2).

Sex and period had statistically significant effects on the $\delta^{13}C$ of the bone of the skulls collected in Chubut Province (Table 4). The absence of a significant interaction term in the 2-way ANOVA (sex × period) indicates that males and females have responded in the same way to the changes in northern Patagonia since the 1940s. Post hoc tests conducted independently for each sex (males ANOVA: $F_{3,36} = 18.849$, $p < 0.001$; females ANOVA: $F_{3,31} = 4.790$, $p = 0.007$) demonstrated that the $\delta^{13}C$ of both males and females increased from the first to the second period and that differences did not exist between the first and the fourth period for either sex (Fig. 4a,b).

The linear regression analysis of the values for skulls from the CENPAT collection confirmed that bone $\delta^{13}C$ has decreased steadily in both sexes since the 1970s (Fig. 5), and that the magnitude of the decline was the same in both sexes, because although the regression equations were different, they resulted in 2 parallel lines (test of coincidence: $F_{2,56} = 31.28$, $p < 0.001$; test of parallelism: $t = 0.572$, df = 56, $p = 0.550$). Considering the

above-reported differences in the $\delta^{13}C$ of potential prey, both males and females are thought to have reduced the consumption of pelagic prey from the 1940s to the 1970s and to have increased it again since the 1970s.

Period was the only factor with a statistically significant effect on the $\delta^{15}N$ of the bone of the skulls collected in Chubut Province, as the sex and interaction terms of the 2-way ANOVA (sex × period) were not statistically significant (Table 4). Post hoc tests conducted independently for each sex (males ANOVA: $F_{3,36} = 1.685$, $p = 0.188$; females ANOVA: $F_{3,31} = 7.360$, $p < 0.001$) revealed that the female skulls from the first period were more enriched in ^{15}N than those from the other periods and there was no difference in the $\delta^{15}N$ of male skulls in the 4 periods considered (Fig. 4c,d). Such a conclusion is reinforced by the absence of correlation between the stranding year and the $\delta^{15}N$ of female

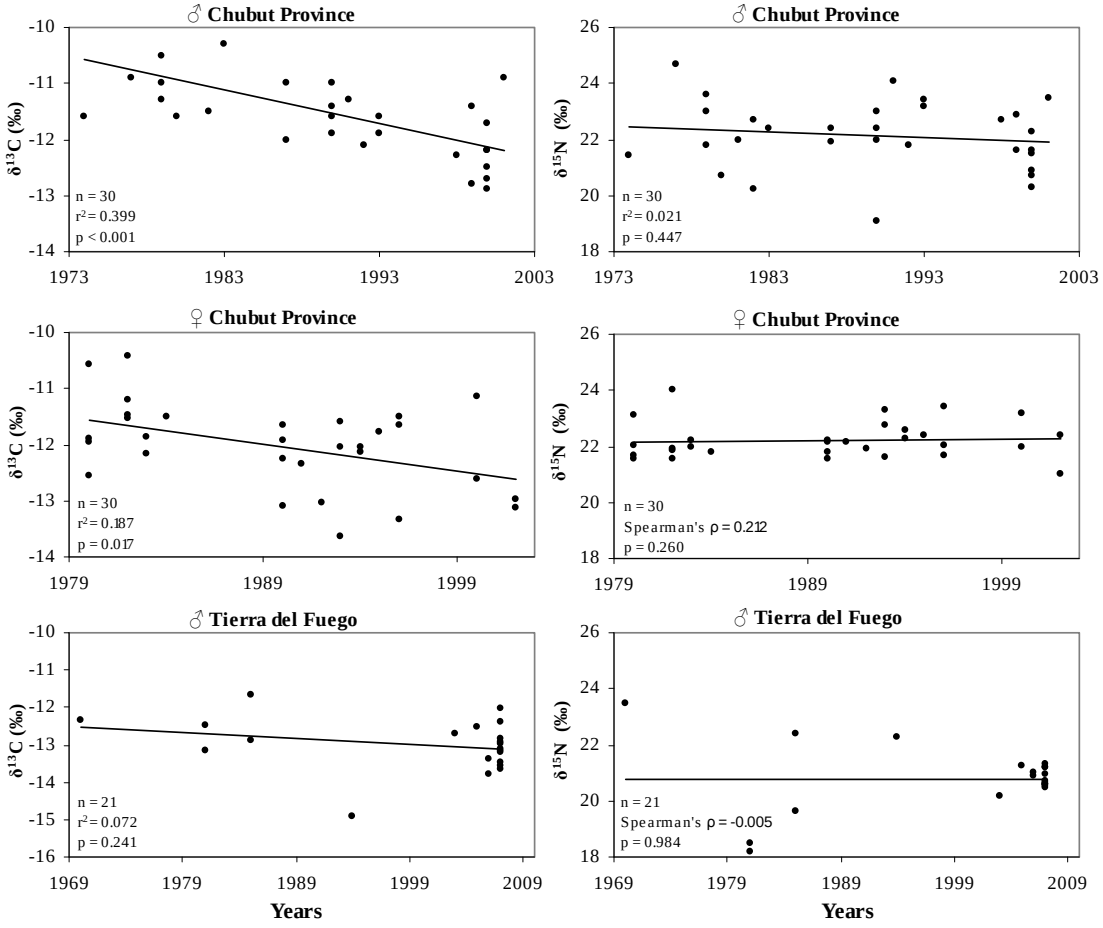

Fig. 5. *Otaria flavescens.* Linear regression and non-parametric correlation between the year of stranding and the isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) of skull bone of adult male and female South American sea lions from the CENPAT collection (Chubut Province) and adult males from the Acatushún Museum (Tierra del Fuego)

skulls from the CENPAT collection (Fig. 5). The same was true for the $\delta^{15}N$ of the males (Fig. 5). These results suggest that males also increased the consumption of those benthic prey enriched in ^{15}N, like octopuses, in parallel to the consumption of pelagic prey, thus stabilizing the $\delta^{15}N$ of skull bone. The same was true for the females, except in the 1940s.

The analysis of male skulls from the Acatushún Museum (Tierra del Fuego) revealed no change with time either in $\delta^{13}C$ or in $\delta^{15}N$ values (Fig. 5). This result is unlikely to be an artefact caused by a small sample size or an uneven temporal distribution of samples, as indicated by the randomisation exercise carried out with the samples from Chubut Province. This exercise was conducted to test the influence of a sample size and a temporal distribution of samples like that of the Acatushún Museum collection on the capacity to detect the existence of a statistically significant negative correlation between stranding year and the $\delta^{13}C$ of bone. The result of the exercise was that such a correlation was detected in 98% of the randomised populations, thus indicating a probability of not identifying a correlation when it exists of only 2%, equivalent to a p-value of 0.02.

DISCUSSION

The energy density of prey plays a central role in the foraging ecology of at least some otariids (Rosen & Trites 2000, Staniland et al. 2007). The energy density of pelagic potential prey for sea lions *Otaria flavescens* off Chubut Province is much higher than that of the benthic potential prey (present study), so sea lions might be expected to show a preference for pelagic prey if energy density were the only criteria for prey selection. In such a scenario, the per capita consumption of pelagic prey would be expected to have increased from the 1940s to the 1970s, due to the resulting reduction in intraspecific competition caused by sealing and the concomitant reduction in the population of sea lions (Fig. 2b). Conversely, the per capita consumption of pelagic prey would be expected to have decreased since the 1970s, due to the development of industrial fishing targeting pelagic species, the decline of the hake stock (Fig. 2a) and the partial recovery of the sea lion population (Fig. 2b).

The isotopic data presented here do not allow a precise reconstruction of the composition of diet of South American sea lions since the 1940s, because prey-to-predator

fractionation factors have not been determined experimentally for this species. Furthermore, the fractionation factors determined experimentally for other pinniped species (Hobson et al. 1996, Kurle 2002) are for tissues other than bone, and, due to the high divergences observed among tissues, any diet reconstruction based on them will be meaningless. However, the isotopic data presented here are useful in detecting changes in the relative contribution of benthic and pelagic prey to the diet of South American sea lions.

If the relative contribution of Argentine hake and other pelagic prey to the diet of South American sea lions had decreased in response to the development of industrial fishing, the $\delta^{13}C$ of skull bone would have had to increased. The changes in the $\delta^{13}C$ of skull bone reported here follow exactly the opposite pattern (Fig. 2c), so the current diet should be viewed as the result of a shift from a benthic-based diet in the 1970s to a current diet based on a large fraction of pelagic prey, mainly Argentine hake. Furthermore, the similarity between the bone isotopic signals of the sea lions stranded in the 2000s and those collected from a hunting site in the 1940s indicate similar diets in both periods (Figs. 2 & 4).

Increased per capita consumption of Argentine hake *Merluccius hubbsi* and other pelagic prey at a time when the hake population was decreasing is a paradoxical result, but it can be explained by 3 independent, non-excluding mechanisms: (1) increased availability of juvenile hake due to cannibalism release, (2) increased consumption of discarded hake by sea lions and (3) increased exploitation of pelagic feeding grounds due to increased intraspecific competition at benthic foraging grounds as the sea lion population grew.

Cannibalism is common in the Argentine hake (Angelescu & Prenski 1987), so increased survival of young size classes might be expected if fishing reduced the number of older specimens (Laevastu & Favorite 1988). This scenario is consistent with the length structure of the hake population in the 1990s, when individuals shorter than 30 cm dominated the population (Bezzi et al. 2004), but nothing is known about the length structure of the hake population in the 1970s.

On the other hand, the Argentine hake *Merluccius hubbsi* and Argentine red shrimp *Pleoticus muelleri* fisheries discarded annually about 170 000 t of undersized (<30 cm) Argentine hake in the late 1990s off northern Patagonia (Crespo et al. 1997, Cañete et al. 2000, Dato et al. 2003b, Aubone et al. 2004). This is precisely the preferred size of the

Argentine hake consumed by South American sea lions (Crespo et al. 1997), and the total biomass of discarded Argentine hake is slightly larger than the biomass expected to be consumed annually by the population of South American sea lions in the same region (152 565 t in agreement with Koen Alonso & Yodzis 2005). Although South American sea lions were certainly unable to locate every discarded hake, discarding probably increased availability.

The hypothesis that South American sea lions have increased their consumption of hake off northern Patagonia because of increased intraspecific competition for benthic resources (Crespo & Pedraza 1991) is supported by the opposing changes in isotopic signal and population experienced since the 1940s (Fig. 2b,c). Further support comes from the higher nutritional value of pelagic prey (present study), which may compensate for longer foraging trips when exploiting off-shore, pelagic feeding grounds (Staniland et al. 2007). A third line of evidence is the unexpected absence of differences between the isotopic values of males and females both in the 1940s and in the 2000s. South American sea lions are highly dimorphic, with males doubling females in weight (Cappozzo 2002). Dive time and dive depth in pinnipeds depend on oxygen stores and, hence, on body mass (Costa et al. 2004), which often result in males of highly dimorphic species diving deeper and/or longer than females (Le Boeuf et al. 1996, 2000). The data reported here were consistent with a more intense use of benthic resources by males in the 1970s and the 1990s, but not in the 1940s and the 2000s, when the isotopic signal of both sexes was indistinguishable and consistent with a pelagic-based diet for both sexes.

Comparison with the feeding habits of South American sea lions in other regions reveals the relevance of industrial fishing in the above-reported changes. South American sea lions off central Chile, for instance, have also been reported to have shifted recently to a more pelagic diet (Hückstädt & Antezana 2003, Hückstädt et al. 2007), due to the development of a purse-seine fishery targeting pelagic fish (Hückstädt & Antezana 2003) and increased accessibility to pelagic prey. Conversely, the isotopic signal of male South American sea lions from Tierra del Fuego, where commercial fishing is scarce, have probably not changed in the past 3 decades (present study), although this should be considered a preliminary conclusion due to the small sample size and the uneven temporal distribution of samples. However, the apparent absence of changes reinforces the

hypothesis that the increased consumption of Argentine hake off northern Patagonia is a local phenomenon caused paradoxically by the development of industrial fishing.

However, a number of confounding factors may interfere with the interpretation of the temporal change in the isotopic signal of the sea lion skull bone and have to be ruled out before concluding that sea lions have actually increased their per capita consumption of Argentine hake. The first of those factors is the invasion of Chubut Province by the $\delta^{13}C$-depleted benthic macrophyte *Undaria pinnatifida*, first detected in Argentina in 1992 and currently one of the dominant species in the subtidal assemblage of benthic macrophytes off the Chubut Province (Casas et al. 2004). The arrival of *U. pinnatifida* to the coast off northern Patagonia might have caused a drop in the $\delta^{13}C$ of the whole food web and, hence, a decrease in the $\delta^{13}C$ of the skulls of the local sea lions. However, *U. pinnatifida* was recorded for the first time off Chubut Province in 1992, well after the beginning of the decline of the $\delta^{13}C$ in the skulls of South America sea lions.

The second possible confounding factor is a general change in surface ocean carbon reservoirs, due to the increasing concentrations of ^{13}C-depleted atmospheric CO_2 as a consequence of fossil fuel burning, a phenomenon invoked by Cullen et al. (2001) and Newsome et al. (2007) to explain the decline of the $\delta^{13}C$ values of several species from the western Gulf of Alaska and eastern Bearing Sea throughout the second half of the 20th century (but see Schell 2000). In such a scenario, the $\delta^{13}C$ of the sea lions from Tierra del Fuego was also expected to decrease, but it remained stable. The northern Patagonia dataset failed also to reveal a pattern of steady decline consistent with the Suess effect (Cullen et al. 2001), because the $\delta^{13}C$ values of the bone from the skulls increased from the 1940s to the 1970s and then declined.

Cyclical changes in the primary production of the southwestern Atlantic are a third possible confounding factor. Primary production in the southwestern Atlantic is influenced by El Niño Southern Oscillation (ENSO) and the periodic rotation of the subtropical anticyclone (Venegas et al. 1996, Fiedler 2002). A reduction in primary production is known to reduce the $\delta^{13}C$ of primary producers and therefore the isotopic values of the entire food web (Bidigare et al. 1997), so the above-reported decline in the $\delta^{13}C$ of sea lion skull bone might just reflect the periodicity of those climatic processes if samples were collected in periods of contrasting productivity. However, this is an unlikely explanation,

as the ENSO and the displacement of the subtropical anticyclone have a cyclic frequency of 4 and 15 yr, respectively, and the skull dataset from northern Patagonia covers 30 yr with evenly spaced samples.

Conversely, the Pacific Decadal Oscillation (PDO) may have contributed to the observed variability, although the possible links between PDO and productivity in the southwestern Atlantic are obscure. The typical period of the PDO is over 20 to 30 yr, and the last warm phase lasted from 1976 to 2000 (Mantua & Hare 2002), thus overlapping with the northern Patagonia dataset (1974 to 2002). Interestingly, the sea lions stranded dead in northern Patagonia earlier than 1982 may have experienced the effects of the last years of the 1947 to 1975 cold phase of the PDO and those stranded dead in 2001 and 2002 may have experienced the effects of a new cold phase just for a short period before death. The sea lions sloughed at the hunting site at Península Valdés in the 1940s may have also experienced the transition from a warm (1925 to 1946) to a cold (1947 to 1975) phase (Mantua et al. 1997) and, hence, might have experienced environmental conditions like those of the sea lions stranded dead in northern Patagonia in the late 1990s and early 2000s. Remarkably, the $\delta^{13}C$ values of the sea lions collected in the 1940s and those stranded dead in the late 1990s and early 2000s are similar and differ from those of the sea lions stranded dead in the late 1970s.

Furthermore, the dataset from northern Patagonia spans over a period of steady changes in a number of atmospheric parameters, such as the anomalies of the global air temperature, the atmospheric circulation index and the atmospheric CO_2 measured at Mauna Loa (Chavez et al. 2003). Changes in these parameters are known to be related with multidecadal changes in ecosystem dynamics in the Pacific Ocean (Chavez et al. 2003), and the anomalies of the global air temperature and atmospheric circulation index in the 1940s were similar to those in the late 1990s. As a consequence, the changes in the isotopic signal reported here from northern Patagonia are consistent with a possible regimen shift in the southwestern Atlantic related to the regimen shift in the Pacific Ocean reported by Chavez et al. (2003), although the dataset form Tierra del Fuego revealed no major changes in the region. Nevertheless, the possible causal mechanism of such a hypothetic regime sift in the southwestern Atlantic is unknown, and no other evidence exists to our knowledge. As a consequence, increased consumption of pelagic prey, mostly hake, by

South American sea lions off the Chubut Province stands as the most likely hypothesis consistent with the patterns of isotopic change reported here, a result demonstrating the capacity of South American sea lions to recover their original role in the food web despite the profound changes in the ecosystem caused by human activity.

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SECCIÓN 6.

LA REDUCCIÓN DEL TAMAÑO DEL CRÁNEO EN LOS LEONES MARINOS DEL SUR REVELA UN CRECIMIENTO DENSODEPENDIENTE DURANTE LA RECUPERACIÓN DE LA POBLACIÓN

RESUMEN: Se han utilizado datos biométricos procedentes de una colección de cráneos de león marino del sur recolectados en el norte de la Patagonia entre 1974 y 2007 para evaluar la hipótesis de que el crecimiento somático de los leones marinos en dicha región se ha visto afectado por la recuperación de la población tras el cese de la explotación comercial, así como por el desarrollo de la pesquería industrial dirigida a la merluza argentina. El período considerado incluye tres épocas muy diferentes en cuanto a la disponibilidad de alimento para los leones marinos, debido a las variaciones registradas en el tamaño poblacional y en el volumen de desembarques pesqueros. La magnitud de la mayor parte de las diecinueve variables craneométricas consideradas disminuyó a lo largo del tiempo en ambos sexos, aunque de forma más acusada en las hembras. La misma tendencia se observó para el valor de la variable extraída mediante análisis de componentes principales y que explicaba la mayor parte de la variabilidad observada, estando relacionada dicha variable con el volumen del cráneo. Ahora bien, esta reducción del volumen craneal no fue constante en el tiempo, pues sólo se observaron diferencias estadísticamente significativas entre el valor de dicha variable en los leones marinos del período más reciente y los de los dos períodos anteriores. Este último período ha estado marcado por una recuperación de la población de leones marinos y por el colapso de la pesquería de merluza, lo que sugiere una menor disponibilidad de alimento *per capita*. Esta interpretación se ve apoyada por el valor significativamente menor de dicha variable volumétrica con respecto a los otros dos períodos anteriores. Dada la estrecha relación existente entre el tamaño del cráneo y el tamaño corporal, los datos anteriores indican que el crecimiento somático de los leones marinos ha disminuido al incrementarse la densidad de población y desarrollarse la pesca industrial.

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Reduction of skull size in South American sea lions reveals density-dependent growth during population recovery

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ABSTRACT: Craniometrical data of male and female skulls collected from 1974 to 2007 were used to test the hypothesis that the somatic growth of South American sea lions in northern Patagonia has been affected by a reduction in the *per capita* food availability due to combination of the population recovery after sealing cessation and the development of industrial fishing targeting Argentine hake. Most of the nineteen craniometric considered variables decreased through time in both sexes and the same trend was found for a variable extracted by means of principal component analysis and related to skull volume. Most of the reductions in skull size and volume happened since 1990, when the sea lion population peaked and the hake population collapsed. This evidence, combined with a review of supplementary data derived from stable isotope analysis, supports the hypotheses that the somatic growth of South American sea lions is density-dependent and that industrial fishing has reduced the carrying capacity of the ecosystem for South American sea lions.

KEY WORDS: density-dependent growth; somatic growth; overfishering; craniometry; South American sea lion; *Otaria flavescens*; skull size; Argentine hake.

INTRODUCTION

Density dependence is at the very core of the classic population ecology (Rockwood 2006) but testing it in the wild for marine vertebrates has been extremely difficult. Although in some cases populations are actually well below carrying capacity and growth and demographic parameters are independent of population size (Doherty 2002), the most common situation is the inability of researchers to carry out the experiments required to test the predictions of density dependence (Hixon & Webster 2002). Nevertheless, the past and present massive exploitation of large marine vertebrates (Myers & Worm 2003,

Lewison et al. 2004) offers the possibility of studying the response of these species to dramatic changes in population size. Changes in demographic parameters will often be impossible to document in the absence of data about the pre-exploitation period, but museum collections offer an opportunity to test predictions about the impact of population size on somatic growth.

Many otariid species were hunted to the brink of extinction throughout the 19th and 20th centuries due to commercial exploitation for their pelts and fat (Bonner 1982). After killing cessation, some species quickly recovered but others failed to do so, mainly in the Southern Ocean and adjoining regions (Trites 1992, Wickens & York 1997, Gerber & Hilborn 2001, National Research Council 2002, Costa et al. 2006). At least one of these species, the northern fur seal (*Callorhinus ursinus*), exhibited strong density-dependent somatic growth during the recovery period that followed hunting cessation (Scheffer 1955, Etnier 2004). A similar pattern has been suggested for male Antarctic fur seal (*Arctocephalus gazella*) at South Georgia, on the basis of the reduced width of tooth annulus in recent individuals (Hanson et al. 2009). On the other hand, reduced body size in Steller sea lions (*Eumetopias jubatus*) through the 1980s has been hypothesised to be the result of nutritional stress caused by changes in the ecosystem (Trites & Donnelly 2003).

The South American sea lion (*Otaria flavescens*) was heavily exploited in the south-western Atlantic Ocean from the 1920s to the 1960s (Godoy 1963), where most populations had been reduced to less than 10% of their original number when exploitation ceased (Crespo & Pedraza 1991, Reyes et al. 1999, Schiavini et al. 2004). In Argentina, the recovery of the population only began in the early 1990s, after three decades of stagnation (Crespo & Pedraza 1991, Reyes et al. 1999, Schiavini et al. 2004), and now the population is about one third of the original size (Reyes et al. 1999, Dans et al. 2004, Schiavini et al. 2004). Drago et al. (2009a) reported an increased reliance of South American sea lions on off-shore, pelagic prey since the 1970s, probably because an increased intraspecific competition at benthic foraging grounds, as the population grew, forced them to exploit more distant foraging grounds. Furthermore, Drago et al. (2009a) pointed out that massive fish discards by bottom trawlers, mainly undersized Argentine hake (*Merluccius hubbsi*), may have resulted in increased accessibility to this particular demersal pelagic species. Although pelagic potential prey have a higher nutritional quality than benthic potential

prey (Eder & Lewis 2005, Drago et al. 2009a), the stock of Argentine hake has declined dramatically since the development of industrial fishing (Lloris et al. 2003, Koen Alonso & Yodzis 2005) and the Magellanic penguins (*Spheniscus magellanicus*), a potential competitor for small schooling fishes (Thompson 1989, Thompson et al. 1998, Schiavini et al. 2005), are now more abundant than ever (Carribero et al. 1995, Schiavini et al. 2005). All of these aforementioned factors raises some doubts about the future trends of the South American sea lion population, because the carrying capacity for South American sea lions in this resulting new, highly anthropogenized ecosystem might be much lower than it used to be before the development of industrial fishing.

Assessing changes in the body size of South American sea lions throughout the 20th century is one possible way of understanding the correlations between somatic growth and the effects of limiting factors such as population density and prey availability, since mammal adult body size is positively correlated with food availability before puberty (Scheffer 1955) and body size is highly correlated with skeletal measurements in otariids (Scheffer 1950, Scheffer & Wilke 1953, Rosas et al. 1993, Miller et al. 2000). Furthermore, skeletal measurements are easily standardized and thereby measurement errors are minimized.

This study investigates how population recovery after sealing and the development of industrial fishing has affected the South American sea lion size in northern Patagonia. The hypothesis to be tested is the existence of an inverse correlation between somatic growth and population density because of decreasing in per capita food availability.

MATERIALS AND METHODS

Sampling. A total of 194 skulls of South American sea lions (92 males and 102 females) from the scientific collection of the Centro Nacional Patagónico (CENPAT) at Puerto Madryn, Argentina, were examined to determine size changes during the last four decades. All the skulls had first been cleaned by dermestid beetles, then boiled to eliminate the remaining fat and flesh, washed with water and soap and finally stored dry. The skulls taken into consideration belonged to specimens that had been accidentally captured by fishermen or stranded dead along the coast of the heavily fished area off the Chubut province, in northern Patagonia (Fig. 1) from 1974 to 2007. This time window included

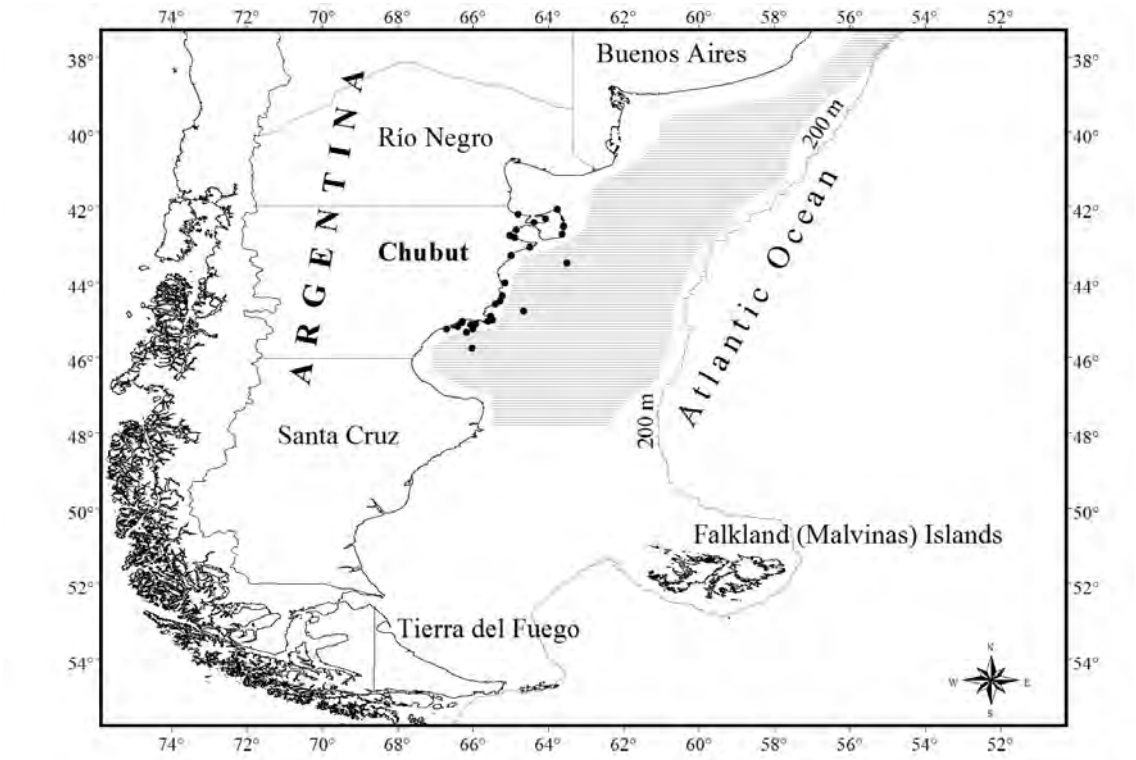

Fig. 1. Atlantic coast of Argentina and location of the Chubut province. The solid black circles (●) show the sampling location of South American sea lions. The hatching shows the fishing grounds exploited by the Argentine industrial fishing fleet in the second half of the 1980s and in the 1990s (Giangiobbe et al. 1993, Dato et al. 2003).

three time periods of contrasting availability of the principal prey (Argentine hake) for South American sea lions in the area (Koen Alonso et al. 1996, Bertolotti et al. 2001, Dans et al. 2003, Lloris et al. 2003, Koen Alonso & Yodzis 2005) (Fig. 2a,b). Actually the 1960s and 1970s (1960-1980) were characterized by high hake availability, as indicated by a very large estimated hake biomass and a very small population of the South American sea lion. After the 1970s (1981-1997) hake availability began to decline, as indicated by the trend of estimated hake biomass. The latest years (1998-2002) were marked by a recovery of the South American sea lion population as well as by the collapse of the hake fishery.

Skull size is highly correlated to body size in South American sea lions (Rosas et al. 1993) and remains almost invariable after 7-8 years of age for males and 5-7 years of age for females (Rosas et al. 1993, Drago et al. 2009b, Grandi et al. 2010). Hence only skulls from adult individuals of known age were used to avoid any age-related bias. The specimens were aged by counting growth layers in the dentine of the canines (Crespo 1988, Crespo et al. 1994) and the age of the selected individuals ranged from 8 to 19 years

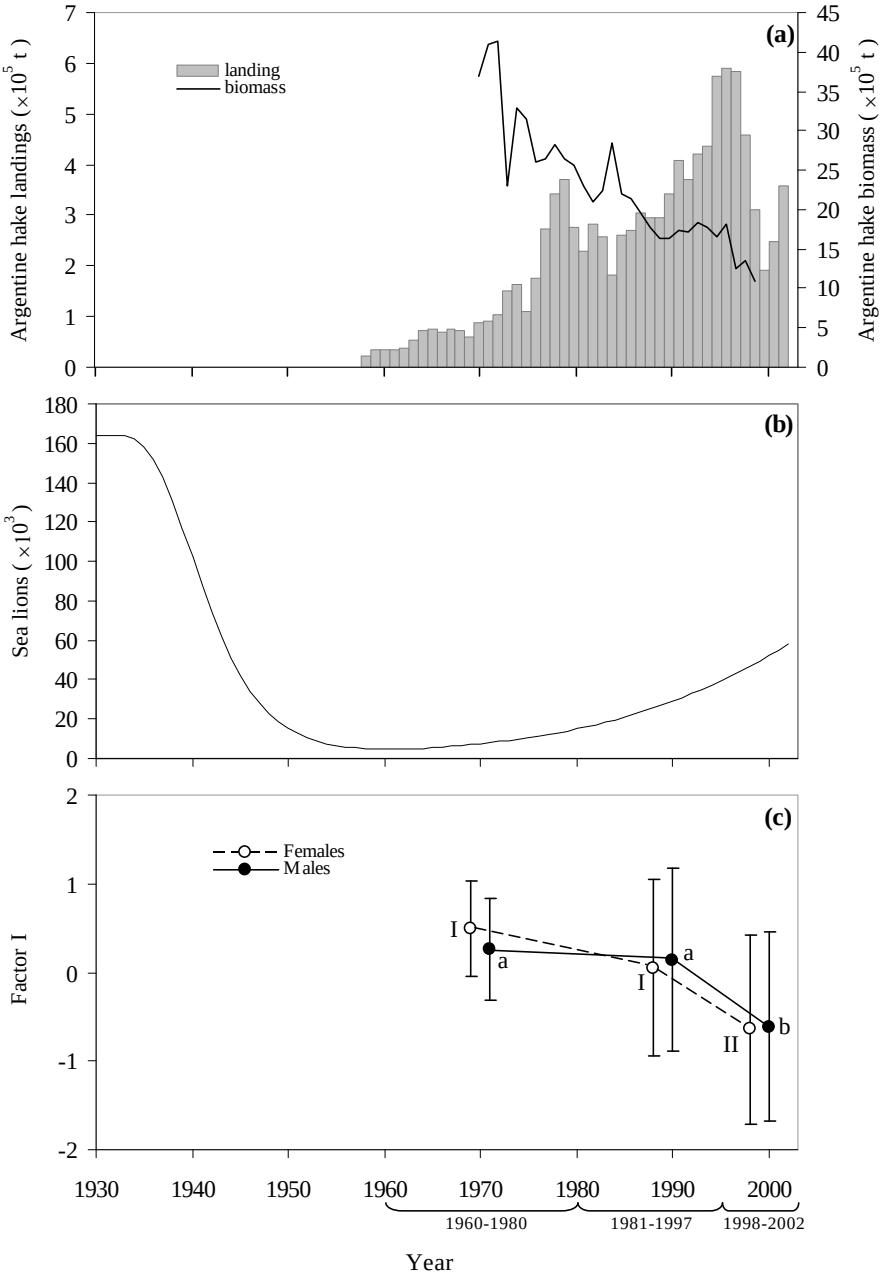

Fig. 2. (a) Hake landings (from Dans et al. 2003) and hake biomass (from Koen Alonso and Yodzis 2005); (b) Trajectory of the sea lion population in Northern and Central Patagonia (from Koen Alonso et al. 1996); (c) Trajectory of the average Factor I related to the skull volume of female and male sea lions from Chubut Province in three contrasting periods (1960-1980; 1981-1997; 1998-2002). Periods within each sex with different letters (males) or Roman numerals (females) differ in their mean values according to the Student-Newman-Keuls post hoc test. Sample size: n = 16, 57, 17 for first, second and third period females respectively; n = 15, 38, 15 for first, second and third period males respectively. The year associated with each Factor I value is the median of each sampling period. Error bars: SD.

for males and from 7 to 20 years for females. Errors in age determination are assumed to be similar through the considered time window and should not bias the results significantly. Nineteen linear measurements were made on the cranium and mandible of each skull (Fig. 3) with calipers (accuracy ± 0.1 mm) by only one person (M. Drago) to avoid interobserver errors. These measurements were selected according to the most influential dimension variables for the two pinniped species considered by Amano et al. (2002) and Brunner (2002). The standard body length (SL) of only 49 specimens (20 males and 29 females) collected from 1990 to 2006 was also measured and used to evaluate any change in the relationship between skull length (condylobasal length or CBL) and body

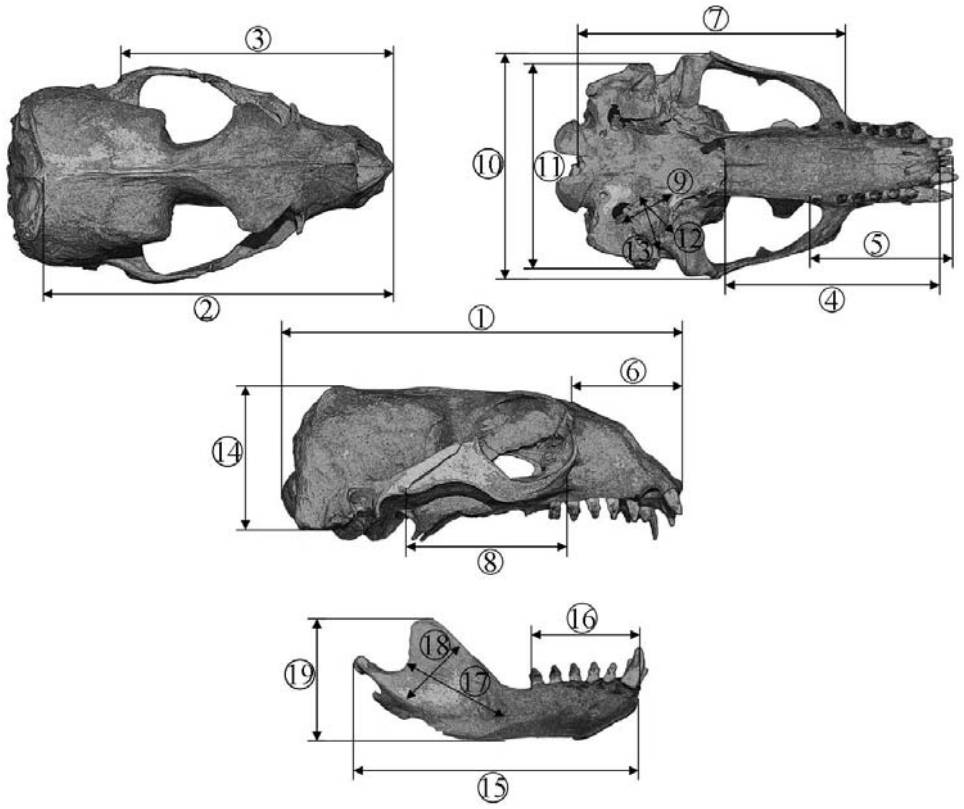

Fig. 3. Dorsal, ventral and right lateral perspectives of the adult female South American sea lion skull showing measurements taken in this study: 1, condylobasal length (CBL); 2, gnathion-to-occipital crest middle length (GOCL); 3, gnathion-to-caudal postglenoid process border length (GPPL); 4, palatal length (PL); 5, maxillary tooth row length (MaxTL); 6, snout length from posterior nasal margin (SL); 7, basion-to-zygomatic root length (BZRL); 8, zygomatic length (ZL); 9, bulla length (BL); 10, zygomatic width (ZW); 11, mastoid width (MW); 12, bulla average width (BAW); 13, bulla maximum width (BMW); 14, cranium height (CH); 15, mandible length (ML); 16, mandibular tooth row length (ManTL); 17, masseteric fossa length (MFL); 18, masseteric fossa width (MFW); 19, mandible height (MH).

length. SL was measured according to standard methods of the Committee on Marine Mammals (1967).

Data analyses. Males and females were analyzed separately because of the marked sexual dimorphism of South American sea lions (Rosas et al. 1993, Sanfelice & De Freitas 2008, Drago et al. 2009b). The stability of the CBL/SL ratio through time within each sex was tested by linear regression analysis, whereas the influence of birth year on each craniometrical variable was tested using the non-parametric Spearman ρ correlation coefficient. Principal component analysis (PCA) was used to investigate the sources of morphological variation within each sex and to reduce the number of original variables to a few principal components only. Prior to PCA the values for the original variables had been standardized, so that each variable had equal weighting and bias of larger measurements dominating the smaller ones were removed. Extracted components were ordered in terms of magnitude of their variances. A non-parametric Spearman ρ correlation coefficient was used again to test the correlation between the birth year and the factor score extracted by PCA to investigate the time trends of different dimensional facets in the skull of adult male and female sea lions.

The birth year of each specimen was calculated by subtracting the age at death from the stranding year. The year when somatic growth finished was calculated by adding 8 years to the birth year of males and 6 years to the birth year of females (Rosas et al. 1993, Drago et al. 2009b, Grandi et al. 2010). The sea lions were allotted within the aforementioned time periods so that the growth span of each individual spanned only one of the three different periods in which food availability was considered to be dissimilar. When the growth span included two time periods, the individual was assigned to the time period which encompassed the longest part of its growth span. The only individual whose growth span covered equally two periods (one of the females in the third period) was assigned to the most recent one. One-way ANOVA, followed by the Student-Newman-Keuls post hoc test, was used to compare the principal factor score, extracted by PCA, of individuals of the same sex in the three considered periods.

Data are always shown as mean ± standard deviation (SD), unless otherwise stated. All statistical analyses were conducted with the SPSS 15 software package. Prior to any

statistical analysis, normality in data distribution was tested by a Lilliefors's test and their homogeneity of variances by a Levene's contrast test.

RESULTS

Birth year was statistically and negatively correlated with most of the analyzed craniometrical variables of females (Table 1). The most remarkable exception was the length of the tympanic bulla (variable # 9), which increased with the birth year (Table 1). The birth year was also statistically and negatively correlated with the craniometrical variables of males related to the cranium width and height and also to the mandible length and height (Table 1). The variables related to the cranium length were uncorrelated with the birth year though two of them, the GPPL (variable # 3) and BZRL (variable # 7), were on the verge of significance. The same was true for the MFL (variable # 17) related to the mandible length (Table 1).

Table 1. Results of the non-parametric Spearman ρ correlation between the birth year and the craniometrical variables of adult male and female South American sea lions. Sample size (n), correlation coefficient (ρ), significance level (p). Statistical significance at p-value < 0.05 in bold. For variable numbers and abbreviations, see Fig. 3.

Skull region	Dimension	Variable		♀			♂		
		No.	Abbr.	n	ρ	p	n	ρ	p
Cranium	**Length**	1	CBL	99	-0.375	**< 0.001**	85	-0.135	0.217
		2	GOCL	99	-0.307	**0.002**	85	-0.086	0.434
		3	GPPL	99	-0.395	**< 0.001**	85	-0.212	0.052
		4	PL	99	-0.324	**0.001**	85	-0.154	0.159
		5	MaxTL	99	-0.147	0.146	85	-0.008	0.941
		6	SL	98	-0.215	**0.033**	84	-0.149	0.176
		7	BZRL	99	-0.243	**0.015**	85	-0.187	0.086
		8	ZL	99	-0.435	**< 0.001**	85	-0.154	0.160
		9	BL	99	0.344	**< 0.001**	86	-0.069	0.530
	Width	10	ZW	99	-0.501	**< 0.001**	86	-0.461	**< 0.001**
		11	MW	99	-0.322	**0.001**	86	-0.339	**0.001**
		12	BAW	99	0.068	0.501	86	-0.124	0.255
		13	BMW	99	-0.284	**0.004**	86	-0.291	**0.007**
	Height	14	CH	99	-0.051	0.616	86	-0.270	**0.012**
Mandible	**Length**	15	ML	94	-0.412	**< 0.001**	74	-0.259	**0.026**
		16	ManTL	94	-0.124	0.232	74	0.005	0.969
		17	MFL	94	-0.352	**< 0.001**	76	-0.204	0.078
	Width	18	MFW	94	-0.160	0.123	75	-0.078	0.505
	Height	19	MH	94	-0.351	**0.001**	75	-0.318	**0.005**

PCA extracted three components for females' skulls and two for males' skulls (Table 2). Component I accounted for 55.6% of the total variance for females' skulls and 64.4% of the total variance for males' skulls and was related to the skull volume in both sexes, as variables concerning the three spatial dimensions had a similar weight (Table 2). Component II accounted for a further 10.0% of the total variance for females' skulls and 7.6% for males' skulls and was primarily affected by variables concerning skull length (Table 2). Component III, significant only for females' skulls, accounted for another further 5.4% of the total explained variance but was obscure in its interpretation.

The birth year was negatively and significantly correlated with the factor score of Component I (Factor I) in both sexes, positively and significantly correlated with the factor score of Component II (Factor II) for females, but not for males (Fig. 4). The birth year

Table 2. Component loading from principal component analysis for craniometrical variables of adult male and female South American sea lions. For variable numbers and abbreviations, see Fig. 3.

Skull region	Dimension	Variable		♀			♂	
				Component			Component	
		No.	Abbr.	I	II	III	I	II
Cranium	**Length**	1	CBL	0.956	-0.061	0.044	0.947	-0.118
		2	GOCL	0.830	-0.105	0.023	0.786	-0.102
		3	GPPL	0.956	-0.109	0.009	0.944	-0.115
		4	PL	0.922	-0.118	0.036	0.932	-0.170
		5	MaxTL	0.702	-0.310	0.045	0.663	-0.181
		6	SL	0.610	-0.230	0.205	0.716	-0.182
		7	BZRL	0.882	0.140	-0.062	0.916	0.065
		8	ZL	0.820	-0.077	-0.010	0.886	-0.098
		9	BL	0.205	0.821	-0.143	0.450	0.721
	Width	10	ZW	0.795	-0.070	0.024	0.870	0.007
		11	MW	0.840	0.088	-0.064	0.857	0.114
		12	BAW	0.247	0.724	0.544	0.539	0.603
		13	BMW	0.610	0.356	0.441	0.801	0.327
	Height	14	CH	0.530	0.411	-0.528	0.725	0.236
Mandible	**Length**	15	ML	0.950	-0.127	0.002	0.917	-0.212
		16	ManTL	0.622	-0.208	0.210	0.746	-0.434
		17	MFL	0.680	-0.164	-0.073	0.895	-0.016
	Width	18	MFW	0.603	0.305	-0.298	0.651	-0.003
	Height	19	MH	0.815	0.041	-0.196	0.784	0.111
Percent of total variance explained				**55.6**	**10.0**	**5.4**	**64.4**	**7.6**

was uncorrelated with the factor score of Component III (Factor III) for females (n = 90, ρ = -0.089, p = 0.405). As the Factor I increased with the skull volume and the Factor II decreased with the skull length, these results suggest a general skull volumetric reduction in both sexes and a skull shortening in females as time passed, and confirmed the results of the statistically-significant correlations between the birth year and analyzed craniometrical variables.

Furthermore, one-way ANOVA (females: $F_{2,87}$ = 6.207, p = 0.003; males: $F_{2,65}$ = 4.070, p = 0.022) and a Student-Newman-Keuls post hoc test conducted independently for each sex demonstrated that the Factor I of both males and females decreased from the second to the third period and that differences did not exist between the first and the second period for either sex (Fig. 2c). Therefore in both sexes the skull volume of sea lions from the third period was significantly smaller than that of sea lions from the other two previous periods considered here. As the CBL/SL ratio of both sexes did not change through time from 1990 to 2006 (females: p = 0.467; males: p = 0.089), a reduction in skull size is expected to be associated with a reduction in body size.

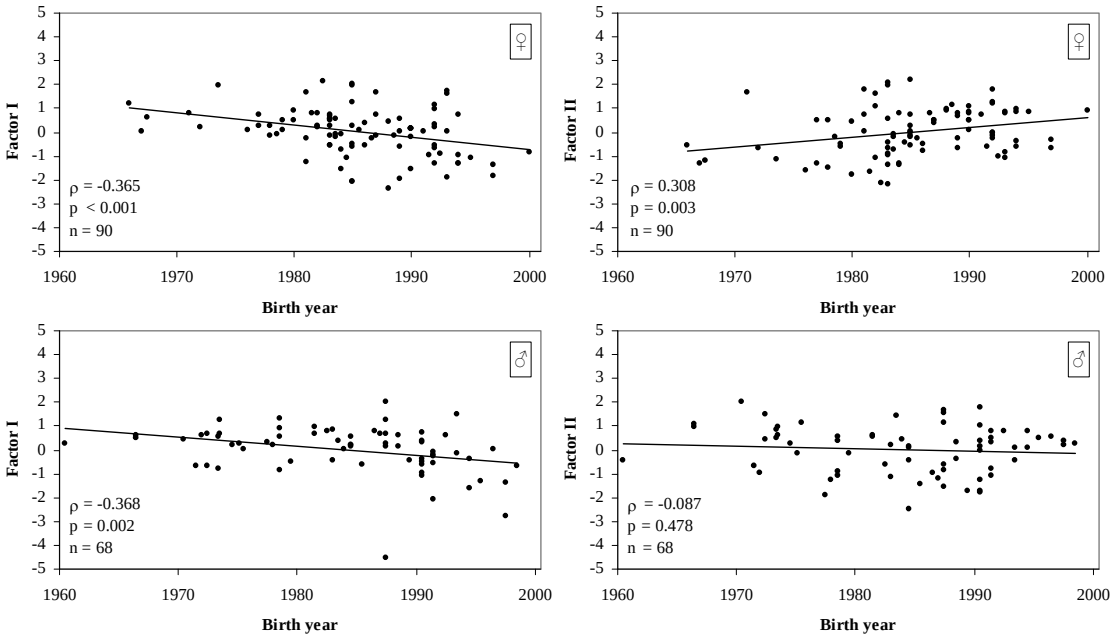

Fig. 4. Non-parametric Spearman ρ correlation between the birth year and the factor score for adult male and female South American sea lions. Sample size (n), correlation coefficient (ρ), significance level (p).

DISCUSSION

The drastic reduction of the South American sea lion population due to commercial sealing was not the only human impact in the ecosystem off northern Patagonia through the 20th century, as in the 1970s a high sea fishery targeting the Argentine hake (*Merluccius hubbsi*) was established there (Crespo et al. 1997, Bertolotti et al. 2001). The development of fishing has dramatically reduced the hake population (Lloris et al. 2003, Koen Alonso & Yodzis 2005), which, combined with the extreme reduction of the sea lion population, has led to a severe reorganization of the whole ecosystem (Koen Alonso & Yodzis 2005). The current ecosystem state is characterized by larger populations of Magellanic penguin (*Spheniscus magellanicus*), Argentine short-fin squid (*Illex argentinus*) and Argentine anchovy (*Engraulis anchoita*) (Carribero et al. 1995, Koen Alonso & Yodzis 2005, Schiavini et al. 2005). As a consequence, some former hake predators, like the spiny dogfish (*Squalus acanthias*), reduced their Argentine hake consumption as the hake stock declined and increased the consumption of alternative prey as their abundance raised (Koen Alonso et al. 2002).

This reorganization of the ecosystem seems not to have prevented the recovery of the South American sea lion population in northern Patagonia, which is currently growing at an annual increase rate of about 6% (Dans et al. 2004). Furthermore, the diet has shifted from a benthic-based diet in the 1970s to a current pelagic-based diet with a high contribution of the Argentine hake (Drago et al. 2009a). Increased consumption of the Argentine hake by the South American sea lion, at a time when the hake population has decreased, is a paradoxical result explained by three independent, non-excluding mechanisms: (1) increased availability of juvenile hake due to cannibalism release, (2) increased consumption of discarded hake by sea lions and (3) increased exploitation of pelagic feeding grounds due to increased intraspecific competition at benthic foraging grounds as the sea lion population grew (Drago et al. 2009a).

Potential pelagic prey for South American sea lions off Patagonia have a higher nutritional value than benthic potential prey (Eder & Lewis 2005, Drago et al. 2009a) and pups whose mothers have a primarily pelagic diet grow faster than those whose mothers have a primarily benthic diet (Drago et al. 2010a). As a consequence, the quality of the South American sea lion diet has probably improved as a result of increased consumption

of pelagic prey since the 1970s (Drago et al. 2009a). However, the results presented here show that the largest average skull size was recorded when the South American sea lion population was at its historical minimum and the somatic growth decreased as the hake stock declined and the South American sea lion population recovered. A similar pattern has been reported for several fur seal species, where the somatic growth and the population level have long been known to be inversely correlated (Scheffer 1955, Etnier 2004, Hanson et al. 2009).

Density dependence of somatic growth is ultimately caused by a reduction in the per capita prey abundance (Trites & Bigg 1992) and hence the skull reduction here reported indicates that the potential improvement in the diet quality caused by a shift to a more pelagic diet has not balanced the reduced per capita prey availability for South American sea lions. The per capita prey availability for South American sea lions may have decreased because of a larger population, a lower availability of the main prey target (hake) due to overfishing, or a combination of both.

South American sea lion pups grew more slowly during early lactation in bigger colonies than in smaller ones, perhaps due to a lower per capita food availability for females during this period (Drago et al. 2010b). Colony size has increased as the population of South American sea lions in northern Patagonia has recovered (Dans et al. 2004), thus leading to a slower growth during early lactation. Under normal circumstances, it would be expected this initial slower growth to be eventually reverted by compensatory growth, a mechanism reported in a wide range of mammalian species, included several pinnipeds (Wilson & Osbourn 1960, Worthy & Lavigne 1983, Kamalzadeh et al. 1998, Eckhardt et al. 2005, Jeanniard du Dot et al. 2009). Therefore, the South American sea lion population should have been able to revert the effects of the slower growth during lactation with a faster post-weaning one, had the availability of offshore, pelagic prey not declined. It is also important to underline that skull size reduction is unlikely to be the result of a loss of genetic variability due to overexploitation, as genetic markers have revealed no population bottleneck (Feijoo M. *unpublished data*). Furthermore, the skull size reduction is observed as occurring starting from the 1990s, thirty years after sealing cessation (Crespo & Pedraza 1991, Reyes et al. 1999) and concomitant to the hake landing peak and the hake stock size decline (Lloris et al. 2003, Koen Alonso & Yodzis 2005). Considering

the skull size reduction observed as occurring in parallel to the population recovery, the overall evidence seems to indicate that industrial fishing may have reduced the carrying capacity of the ecosystem off northern Patagonia for South American sea lions.

Skull size is highly correlated to body size in the South American sea lion (Rosas et al. 1993) and the CBL/SL ratio has remained invariable from 1990 to 2006. This suggests that a reduction in the skull size should have resulted in a parallel reduction in body size. Diving skills in pinnipeds are tightly linked to body size, as larger species exploit deep, benthic habitats, whereas smaller species typically behave as epipelagic predators (Gentry et al. 1986, Kooyman 1989, Costa 1991, 1993, Costa et al. 2004). Similarly, females of some highly dimorphic species have a more pelagic diet than males throughout their life, due to their smaller body size (Le Boeuf et al. 1996, 2000, Meynier et al. 2008). This suggests that the foraging habits of South American sea lions off northern Patagonia may have changed since the 1970s as a result of a decrease in body size.

Stable isotope data indicate that both sexes have become more pelagic since the 1970s (Drago et al. 2009a), in agreement with the foraging behaviour expected from a smaller body mass. The observed increase in length of the tympanic bulla in females might also be related to a higher reliance on pelagic prey in a turbid area as northern Patagonia. This is because a larger tympanic bulla is thought to improve the underwater hearing capacity of pinnipeds and larger bullae are expected in species consuming pelagic fish in turbid water (Hyvärinen & Nieminen 1990).

However, the stable isotope data are also consistent with increased use of off-shore prey due to the depletion of coastal resources caused by a larger population and increased discard of undersized pelagic fish. Stable isotope data also indicate that the current diet of South America sea lions is approaching the one consumed before the collapse of the population and that dietary differences between adult males and females are vanishing (Drago et al. 2009a), which would increase intraspecific competition. Males are probably less affected than females by intraspecific competition, because a larger body mass allows them to dive deeper and stay longer under water. This may explain why the reduction of skull size was less intense in males than in females, although it is unclear why the reduction affected skull width and height but not length. Whatever the reason, in this scenario of reduced carrying capacity due to competition with industrial fishing, the

growth rate of the South American sea lion population in northern Patagonia is expected to decrease in the near future, unless the hake stock rebuilds.

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El león marino del sur *(Otaria flavescens)* fue intensamente perseguido por su piel y grasa. En los años sesenta, al cese de su caza, la población de Patagonia era inferior al 10% de su tamaño original. Este no fue el único impacto humano en el ecosistema marino del Atlántico sudoccidental, ya que simultáneamente al cese de la explotación del león marino se inició la pesca industrial. Esta actividad podría haber afectado la recuperación del león marino no sólo por una posible reducción en la disponibilidad de alimento, sino también por un cambio general en el funcionamiento del ecosistema. Los resultados obtenidos en la presente tesis doctoral revelan, sin embargo, una situación mucho más compleja. La contribución relativa de presas pelágicas de elevada calidad nutricional a la dieta del león marino se ha incrementado en paralelo al desarrollo de la pesca industrial, en vez de disminuir. Además, la población actual de león marino del sur es aproximadamente un tercio de la población original estimada antes de la explotación y crece rápidamente, aunque la talla de los ejemplares adultos se ha reducido en la última década. En este contexto, parece que la pesca puede haber reducido la capacidad de carga para el león marino del sur en la Patagonia, pero eso no tuvo efectos sobre la fase inicial de recuperación demográfica, dado el pequeño tamaño de la población. Únicamente ahora, cuando la población es aproximadamente un tercio de su tamaño inicial, empiezan a observarse los primeros síntomas de una menor capacidad de carga del ecosistema, en forma de una ralentización del crecimiento somático. Si esto es cierto, cabe esperar una disminución de la tasa de crecimiento de la población en los próximos años y una estabilización muy por debajo del tamaño original estimado. Por lo tanto, el estancamiento de la población de león marino observado tras el cese de la explotación no puede atribuirse a una disminución de los recursos alimentarios, ya que la población era pequeña y el impacto de la pesca también. La explicación más probable sería el incremento de la mortalidad de las crías derivada del pequeño tamaño de las colonias de cría y su desestructuración social, lo que habría incrementando la tasa de infanticidio por parte de los machos subadultos, pues en la presente tesis doctoral se ha demostrado la existencia de una relación inversa entre la tasa de mortalidad de las crías y el tamaño de las colonias.