UNIVERSITAT DE BARCELONA

Cambios en la posición trófica del lobo común sudamericano (*Otaria flavescens*) en respuesta a la explotación masiva de mamíferos marinos y pesca industrial

Lisette Zenteno Devaud

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Lisette Zenteno- Tesis Doctoral-2016

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Departamento de Biología Evolutiva, Ecología y Ciencia Ambientales
Programa de Doctorado en Biodiversidad

Cambios en la posición trófica del lobo marino sudamericano (*Otaria flavescens)* en respuesta a la explotación masiva de mamíferos marinos y pesca industrial

Memoria presentada por Lisette Zenteno Devaud
Para optar al grado de Doctora por la Universidad de Barcelona
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CENPAT- CONICET (Argentina)

“I used to think the top global environmental problems were biodiversity
loss, ecosystem collapse and climate change. I thought with 30 years of good
science we could address those problems, but I was wrong.
The top environmental problems are selfishness, greed and apathy - and to
deal with these we need a spiritual and cultural transformation and we
scientists don’t know how to do that”

- Gus Speth

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Contenidos

INFORME DE LOS DIRECTORES

Los directores de tesis, el Dr. Luis Cardona Pascual y el Dr. Enrique A. Crespo certifican que Lisette Zenteno ha realizado las investigaciones contenidas en la presente tesis doctoral titulada "Cambios en la posición trófica de lobo común (*Otaria flavescens*) en respuesta a la explotación masiva de mamíferos marinos y pesca industrial". La tesis consta de 4 trabajos de investigación en formato de artículo científico, tres de los cuales están publicados en revistas científicas internacionales reconocidas en el Science Citation Index (SCI) y el cuarto en preparación. A continuación se detalla la referencia de todos los artículos, indicando el factor de impacto de la revista según el Thomson Institute for Scientific Information, así como la contribución del doctorando en la elaboración de cada artículo.

Primer Capítulo

Zenteno L., Crespo EN., Goodall N., Aguilar A., de Oliveira L., Drago M., Secchi E., Garcia N. & Cardona L (2013). Stable isotopes of oxygen reveal dispersal patterns of the South American sea lion, (*Otaria byronia*), in the southwestern Atlantic. Journal of Zoology 291: 119–126.

Factor de impacto en los últimos 5 años: 2.078

La doctoranda ha participado en el análisis de las muestras, el análisis de los datos, y en la redacción científica.

Segundo Capítulo

Cardona L., Vales D., Aguilar A, Crespo E., Zenteno L (2015)1 Stable isotope values in metabolically inert tissues: more than just dietary changes.

En preparación.

El doctorando ha participado en la recogida y el análisis de las muestras, el análisis de los datos, y en la redacción científica.

Tercer Capítulo

Zenteno L., Crespo E., Aguilar, E., Cardona, L. (2014). Shifting niches of the South American sea lion (*Otaria flavescens*) in the southwestern Atlantic since the late Holocence.

Paleobiology: 41: 387-401. 2015

Factor de impacto en los últimos 5 años: 2,945

El doctorando ha participado en el análisis de las muestras, el análisis de los datos, y en la redacción científica.

Cuarto Capítulo

Zenteno L., Crespo E., Saporiti F., de Oliveira L., Drago M., Cardona L., Secchi ER., Aguilar A. Cardona L. (2015). Dietary consistency of male South American sea lions (*Otaria flavescens*) in southern Brazil during three decades inferred from stable isotope analysis. Marine Biology 162:275–289

Factor de impacto en los últimos 5 años: 2.569

El doctorando ha participado en el análisis de las muestras, el análisis de los datos, y en la redacción científica.

Barcelona, a 14 de abril de 2016

Firmado

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INTRODUCCIÓN GENERAL

“El mar, una vez que te hechiza, te engancha en su
maravillosa red para toda la eternidad”
- Jacques Cousteau

Los recientes avances en la modelación de los ecosistemas marinos y sus pesquerías han permitido identificar los componentes bióticos y abióticos que juegan roles importantes en términos de la estructura y dinámica comunitaria (Christensen & Pauly, 1993; Gaedke, 1995; Thompson et al. 2012; Yen et al. 2016). Con ello se ha comenzado a entender el importante rol de los depredadores en los ecosistemas y los profundos impactos que se producen cuando estos son eliminados (Jackson et al. 2001). Bajo ciertas condiciones, los grandes depredadores pueden ser responsables, en gran medida, de la estructura de los ecosistemas donde habitan. Sus efectos no solo se notan en la abundancia y el comportamiento de sus presas, sino que pueden trasmitirse sucesivamente a través de los niveles tróficos inferiores. La progresión de estos efectos indirectos sobre otros niveles tróficos es lo que se conoce como cascadas tróficas o regulación de arriba hacia abajo (*top down*) (Bowen, 1997; Pauly et al. 1998; Yodzis, 1998; Terborgh & Estes, 2010).

Aunque existen múltiples líneas de evidencia de los efectos de la regulación *top down* (e.g. Estes & Duggins, 1995; Estes et al. 1998, 2009; Myers et al. 2007; O'Connor & Brunno, 2007; Heithaus et al. 2008, 2012) estos estudios son limitados debido a la necesidad de varios años o décadas para evidenciar las respuestas ecológicas a cambios poblacionales. Además, los efectos sobre las funciones e interacciones entre las especies que se generan como resultado de los diferentes niveles de regulación *top down* son mucho más difíciles de detectar y evaluar que los estructurales, por lo que la mayoría de los estudios empíricos sobre las interacciones entre especies se han hecho en especies pequeñas o de movilidad limitada (Estes et al. 2011).

A estas dificultades se agrega el hecho de que las especies de gran tamaño son particularmente vulnerables a los impactos humanos, y muchas de ellas han disminuido en abundancia, o se han extinguido, antes de que exista la posibilidad de estudiarlas y entender su importancia ecológica (Jackson et al. 2001; Martin, 2005; Halpern 2008; Terborgh & Estes, 2010). Como resultado de esta eliminación sistemática de los grandes depredadores, la mayoría de los ecosistemas que observamos hoy en día están regulados por la disponibilidad de nutrientes, es decir de abajo hacia arriba (*bottom- up*) (Baum y Worm 2009).

Muchas veces los estudios no detectan el efecto de la eliminación de los grandes depredadores en el funcionamiento del ecosistema y sólo investigaciones muy detalladas, que generalmente requieren muchos años, permiten entender las complejas relaciones existentes entre los depredadores, sus presas y el ambiente. En este contexto, resulta esencial disponer de un punto de referencia histórico, no sólo para la comprensión de los procesos evolutivos que operaban en ambientes prístinos, sino también para comprender cómo funcionan las interacciones entre los organismos y dar una explicación completa de los factores que regulan los patrones de diversidad biológica y la abundancia de las especies en los distintos niveles tróficos de un ecosistema

Así mismo, un marco de referencia histórico permitirá definir objetivos adecuados para la restauración y gestión de la biodiversidad en una planificación territorial (Jackson et al. 2001; Terborgh & Estes, 2010; Braje & Rick, 2011).

Utilizando este enfoque, algunos estudios han destacado la importancia de los cambios de dieta de los depredadores marinos, a través del tiempo, como resultado de los cambios estructurales de las cadenas tróficas (e.g. Trites et al. 2007; Páez-Rosas et al. 2012) y la interacción con la pesca (e.g. Drago et al. 2009a; Hanson et al. 2009). Sin embargo, esta variabilidad de la dieta es difícil de abordar y rara vez incorporada en los modelos ecológicos a fin de comprender el funcionamiento de los ecosistemas.

La explotación de los pinnípedos en el Atlántico sudoccidental

Los mamíferos marinos como los otáridos, que utilizan la zona costera para reproducción y crianza, son extremadamente sensibles a los efectos de las actividades humanas (Bonner, 1982; Costa et al. 2006). En el Canal del Beagle y el norte de la Patagonia Argentina, el registro zooarqueológico revela una importante explotación de las dos especies de otáridos nativos por los cazadores-recolectores aborígenes durante la segunda mitad del Holoceno (Schiavini, 1993; Orquera & Piana 1999; Gómez Otero, 2006; Favier Dubois et al. 2009; Tivoli & Zangrando, 2011; Borella & Cruz, 2012; Favier Dubois & Scartascini, 2012). A pesar de los numerosos estudios realizados sobre el tema, el efecto de esta explotación sobre las poblaciones de otáridos del Atlántico sudoccidental continua siendo incierto. Los datos recientes demuestran un declive generalizado de la importancia de los pinnípedos en el registro zooraqueológico del Canal del Beagle (Tivoli & Zangrando, 2011), que se ha atribuido tanto a un descenso natural de sus poblaciones debido a la reducción de la productividad oceánica (Saporiti et al.2014), como al impacto directo de la caza aborigen (Zangrando et al. 2014). Aunque ambas hipótesis no son mutuamente excluyentes, desconocemos aún cuál fue el impacto real de la caza aborigen. En cualquier caso, durante los siglos XIX y XX, ambas especies fueron cazadas por los europeos en toda su área de distribución principalmente para el comercio de su piel y grasa (Bonner 1982, Kovacs et al. 2012). Por lo que al lobo marino sudamericano o lobo común (*Otaria flavescens*) se refiere, la especie fue intensamente explotada en todo el litoral argentino desde 1920 (Godoy, 1963). Tras el cese de la explotación comercial, a comienzos de los 70, la población argentina vivió varias décadas de estancamiento, para empezar a recuperarse a comienzos de la década de 1990 (Crespo & Pedraza, 1991; Dans et al. 2004; Schiavini et al. 2004), aunque aún se encuentra muy lejos del tamaño de población original (Grandi et al. 2012).

En las Islas Malvinas/Falkland la pequeña población es actualmente inferior al 1,5% que la original, aunque se está recuperando (Thompson et al. 2005; Baylis et al. 2015). Si bien existen dudas al respecto, en esta zona el declive se atribuye más a un cambio ambiental que a la explotación (Baylis et al. 2015). Finalmente, en Uruguay, la población aún está decreciendo, a pesar del cese de la explotación comercial, lo que estaría relacionado con la relación inversa existente entre el tamaño de las colonias y la supervivencia de las crías (Páez, 2006; Franco-Trecu, 2015).

La conectividad entre las poblaciones de lobo marino sudamericano

Si bien actualmente el lobo marino sudamericano se encuentra ampliamente distribuido (Vaz-Ferreira, 1982, Cappozzo & Perrin, 2009), sus poblaciones se encuentran fragmentadas (Szapkievich et al. 1999; Túnez et al. 2007, 2010; Feijoo et al.2011) como consecuencia no sólo de la disponibilidad discontinua de los recursos, sino también de los efectos de las perturbaciones antropogénicas. En esta situación, los procesos de conectividad y dispersión juegan un papel primordial a la hora de establecer la viabilidad de las poblaciones aisladas, gracias a la inmigración/emigración de individuos que mitigarían el riesgo de extinción y determinarían la viabilidad de las metapoblaciones (Hanski, 1998; Raum-Suryan et al. 2002; Pinsky et al. 2010). Los estudios realizados con marcadores genéticos revelan la existencia de un comportamiento filopátrico por parte de las hembras de esta especie (Szapkievich et al. 1999; Freilich, 2004; Túnez et al. 2007, 2010; Artico et al. 2010; Feijoo et al. 2011). Este comportamiento es bastante común en la mayoría de los mamíferos (Greenwood, 1980) incluido otras especies de pinnípedos (e.g. *Halichoerus grypus* (Pomeroy et al. 1994); *Zalophus wollebaeki* (Wolf & Trillmich, 2007) y *Phoca vitulina* (Harkonen & Harding 2001)), mientras que en los machos de varias especies de pinnípedos, a pesar que también se ha observado la existencia de fidelidad territorial (e.g. *H. grypus* (Pomeroy et al. 2000); *Z. wollebaeki* (Wolf &Trillmich, 2007), la mayoría de las investigaciones señalan una mayor tasa de dispersión por parte estos individuos, principalmente durante la temporada no reproductiva (Riedman, 1990; Raum Suryan et al. 2002). Esta información es coherente con los resultados de marcadores genéticos en lobo marino sudamericano del Atlántico sudoccidental, los cuales revelan un flujo de genes mediado por los machos de la especie (Szapkievich et al. 1999; Freilich, 2004; Túnez et al. 2007, 2010; Artico et al., 2010; Feijoo et al. 2011). También resulta coherente con los resultados de marcadores externos, según los cuales existe una mayor dispersión por parte de los machos adultos, llegando algunos a alcanzar distancias > 700 km después de la estación reproductiva (Vaz-Ferreira, 1982; Rosas et al. 1994; Giardino et al. 2014), mientras que las hembras se mantienen cerca de los sitios reproductivos (Thompson et al. 1998; Campagna et al. 2001). Sin embargo, nada se sabe en relación a las diferencias ontogénicas de la duración de la fase de dispersión.

Cambios históricos en la dieta de los lobos marinos sudamericanos

El lobo marino sudamericano es una especie con una amplia flexibilidad trófica, cuya dieta varía en función de la edad, el sexo y el estado reproductivo (Thompson et al. 1998; Koen Alonso et al. 2000; Oliveira et al. 2008; Drago et al. 2009a; Drago et al.2010a; Romero et al. 2011; Riet-Sapriza et al. 2013; Baylis et al en prensa). Se trata de una especie oportunista con una dieta que se compone de las presas de mayor disponibilidad en la zona donde se encuentra (George-Nascimento et al. 1985, Crespo et al. 1997; Koen Alonso et al. 2000; Naya et al. 2000; Oliveira et al. 2008), incluyendo peces pelágicos, demersales (medianos y pequeños) y mictófidos mesopelágicos, así como también calamares, crustáceos y moluscos (Thompson et al. 1998; Koen Alonso et al. 2000). De esta manera, en la costa del Pacífico, una prácticamente inexistente plataforma continental obliga a los animales a consumir exclusivamente recursos pelágicos (Sielfeld et al. 1997, Hückstädt et al. 2007), mientras que una situación opuesta ocurre en Uruguay y la Patagonia argentina, donde la amplia y llana plataforma continental facilita una dieta dominada básicamente por presas demersales, a pesar de existir una alta variabilidad individual en las áreas de alimentación utilizadas (Koen Alonso et al. 2000; Naya et al. 2000; Oliveira et al. 2008; Riet-Sapriza et al. 2013). Este comportamiento oportunista hace que el lobo común se encuentre en constante conflicto con numerosas pesquerías (Crespo et al. 1997; Koen-Alonso & Yodzis, 2005; Kovacs et al. 2012; Sepúlveda et al. 2007; Riet-Sapriza et al. 2012; Ocampo Reinaldo et al. en prensa).

Los cambios en las tramas tróficas marinas costeras del Océano Atlántico sudoccidental acontecidos durante la segunda mitad del Holoceno como consecuencia de las actividades extractivas humanas (Koen-Alonso & Yodzis, 2005; Saporiti et al. 2014a), junto a los cambios en la ecología trófica del lobo común como consecuencia de su explotación (Drago et al. 2009b) sugieren que esta especie puede haber variado su posición original en las tramas tróficas de la región. En este contexto, estudio recientes realizados en Argentina revelan cambios importantes en los hábitos alimenticios de esta especie a lo largo del siglo XX, los cuales estarían correlacionados tanto con los tamaños poblacionales (competencia intraespecífica) como con el desarrollo de la pesca industrial (Suárez et al. 2005; Drago et al. 2009b; Romero et al. 2011). Sin embargo, la competencia intraespecífica y no la competencia con la pesca, se ha propuesto ser el principal determinante de la composición de la dieta en esta especie (Drago et al. 2009b, 2010b).

Esto sería así porque los lobos marinos sudamericanos se alimentan de presas grandes que capturan cerca del fondo (en lo sucesivo demersales) allí donde la población de lobos es pequeña (Koen Alonso et al. 2000; Drago et al. 2009b; Riet-Sapriza et al. 2013), cambiando a presas pequeñas bentónicas y pelágicas cuando y donde la población de lobos aumenta (Suárez et al 2005; Drago et al 2009b; Romero et al. 2011). Si esta hipótesis es cierta, el actual nicho trófico de los lobos marinos sudamericanos sería diferente al del pasado, cuando la población era mucho mayor y la competencia intraespecífica mucho más intensa (Rodriguez & Bastida 1998; Dans et al. 2004; Schiavini et al. 2004).La dificultad de realizar estudios en especies con ciclos de vida que alternan periodos en tierra y mar han limitado la generación de información biológica sobre las mismas. En el lobo marino sudamericano, la mayor parte de los trabajos de reconstrucción de dieta se han hecho sobre la base del análisis de contenidos estomacales y excrementos (Rosas, 1989; Thompson et al. 1998; Naya et al. 2000; Koen Alonso et al. 2000; Oliveira et al. 2008; Szteren et al. 2004 Romero et al. 2011; Riet-Sapriza et al. 2013) y en menor medida, utilizando marcadores intrínsecos (Drago et al 2009 a, b; Drago et al 2010 a, b).

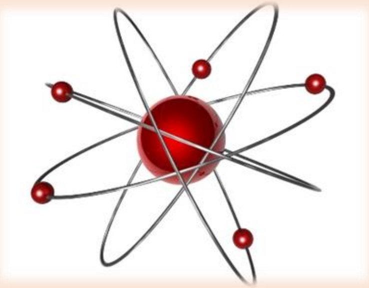
Aunque los métodos tradicionales pueden proporcionar una mejor resolución taxonómica y disminuir la subjetividad al evaluar el papel de las diversas presas, sus resultados deben considerarse como una aproximación relativa, ya que estas técnicas tienen ciertas desventajas que impiden hacer inferencias precisas de la dieta y en particular sobre sus variaciones temporales. Unas de las principales desventajas es el hecho de que la información que se obtiene mediante los métodos tradicionales únicamente representa el periodo entre la ingestión y la digestión del alimento (Petersen & Fry, 1987; Hobson et al. 1996). Además estas técnicas no identifican los componentes que realmente son asimilados por los organismos (Duffy & Jackson 1986) por lo que es posible subestimar la proporción que no pueda ser identificada, en especial a los organismos de cuerpos blandos o las presas que se digieren rápidamente.

Una alternativa que ha sido ampliamente utilizada durante las últimas dos décadas para describir las relaciones tróficas es la abundancia relativa de los isótopos estables del carbono y el nitrógeno (Rubenstein & Hobson 2004; West et al. 2006: Martínez del Río et al. 2009). Esta técnica se basa en que las señales isotópicas de las presas se verán reflejadas en las de sus consumidores, transmitiéndose dichas señales a lo largo de la cadena trófica, desde los productores a los consumidores. Para detectar la contribución de las posibles fuentes de alimento, es necesario únicamente que estas puedan ser distinguidas isotópicamente, y se conozca el factor de discriminación trófica, el cual es el cambio de las razones isotópicas entre los consumidores y sus presas (DeNiro & Epstein, 1978, 1981).

El uso de isótopos estables proporciona ciertas ventajas respecto a los análisis convencionales de dieta, principalmente porque permite conocer los recursos que realmente han sido asimilados y hacer una descripción de la dieta considerando distintos tiempos de integración al utilizar tejidos con diferentes tasas metabólicas (Petersen & Fry, 1987; Hobson et al. 1996; Caut et al. 2009; Silva et al. 2014). Por ejemplo, el colágeno de los huesos es un tejido con baja tasa de renovación que en mamíferos marinos se prevé que promedie la señal de los isótopos estables durante varios años (Riofrío & Aurioles-Gamboa, 2013) y, por lo tanto, es un tejido adecuado para evaluar potenciales cambios en la dieta de un predador durante largos períodos de tiempo (Schoeninger & DeNiro 1984; Lee-Thorp et al. 1989; Hirons et al. 2001a). Por el contrario, los tejidos metabólicamente inertes como las vibrisas, reflejan la dieta al momento de su formación (Hobson, 1999; Cherel et al. 2009; Kernaléguen et al. 2014) y debido a que las vibrisas de los otáridos crecen continuamente, a una tasa constante y no están sujetas a muda (Hirons et al. 2001b), se pueden utilizar para detectar cambios de recursos tróficos y áreas de alimentación durante el lapso temporal representado por el crecimiento de la vibrisa, por lo que estos tejidos permiten reconstruir la historia trófica de un individuo con una resolución casi mensual durante varios años consecutivos (Hirons et al. 2001b; Cherel et al. 2009; Kernaléguen et al. 2012). Este enfoque ha sido utilizado para estudiar los movimientos migratorios (Cherel et al. 2009; Kernaléguen et al. 2012, 2015), los cambios dietéticos ontogenéticos y las diferentes estrategias individuales de alimentación (Lowther & Goldsworthy, 2011; Kernaléguen et al. 2015; Kernáleguen et al. en prensa; Vales et al. 2015; Baylis et al. 2015) en diferentes otáridos.

Sin embargo, la correcta interpretación de los cambios en las razones isotópicas de tejidos inertes requiere información precisa sobre los cambios inducidos por el metabolismo, especialmente sobre los factores de discriminación trófica. En algunos vertebrados, el ayuno tiene una fuerte influencia sobre los valores de los isótopos estables (Hobson et al. 1993; Polischuk et al. 2001; Fuller et al. 2005), mientras que en otras especies no parece tener efecto (Kempster et al 2007; Gómez-Campos et al. 2011; Aguilar et al. 2014). Por ejemplo, algunos estudios han detectado cambios cíclicos de los valores de isótopos estables en tejidos inertes de mamíferos, asociados a la disponibilidad del alimento (Beltrán et al. 2015; Matthews & Ferguson, 2015; Vales et al. 2105), periodos de lactancia (Jenkins et al. 2001; Habran et al. 2010; Cherel et al. 2015; Borrell et al. 2016) y periodos de gestación (Fuller et al. 2004). De ser así, la interpretación del registro isotópico en los tejidos inertes sería más complejo que lo que generalmente se supone.

Isótopos Estables



Los isótopos estables son átomos de un elemento que tienen igual número de protones y electrones, pero diferente número de neutrones (masa atómica) y no sufren decaimiento radioactivo. Estas diferencias en el número másico hace que existan isótopos "pesados" y "ligeros" de un mismo elemento químico (Fry 2006), siendo estos últimos los utilizados preferentemente como substrato de las reacciones químicas, por lo que los residuos que no reaccionan tienden a quedar enriquecidos con el isótopo pesado (Hoeffs, 2004). Las diferencias en la composición isotópica frecuentemente quedan registradas en los tejidos biológicos, y en los perfiles geoquímicos, lo cual constituye el fundamento por el cual el análisis de isótopos estables es una herramienta ampliamente utilizada para reconstruir procesos ecológicos (e.g. West et al. 2006; Koch et al. 1994; Newsome et al. 2010).

La abundancia de los isótopos estables pesados y ligeros en una muestra y en las razones isotópicas es determinada mediante la espectrometría de masas de relaciones isotópicas, donde la razón isotópica de una muestra se expresa en comparación a la de un estándar según la notación δ de la siguiente formula:

$$\delta X = [(R\ \text{muestra} / R\ \text{standard}) - 1] \times 1000\ (‰)$$

siendo X el isótopo de interés (e.g. ^{13}C, ^{15}N, ^{18}O), y R es la abundancia relativa del isótopo pesado en relación al ligero ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$). De esta forma, valores de δX positivos implican que la muestra está enriquecida en el isótopo más pesado en relación con el estándar y valores negativos implican que esta empobrecida (Lajtha &_Michener, 1994).

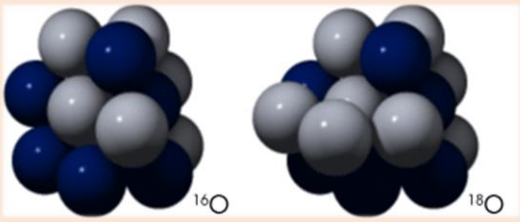
Cuando un organismo consume recursos exógenos, los isótopos estables son asimilados en sus tejidos de acuerdo a la proporción en que se hallan en estos recursos, aunque con cierta diferencia debido a distintos procesos metabólicos. Esta diferencia se conoce como factor de discriminación isotópico y se expresa en la siguiente ecuación:

$$\delta X_t = \delta X_d + \delta \Delta_{dt}$$

siendo t el tejido de interés, d la dieta, Δ_{dt} el factor de discriminación entre la dieta y el tejido. Los valores del factor de discriminación varían en función de la especie, tejido y condiciones ambientales (Gannes et al. 1997; Olive et al. 2003). Sin embargo, debido a que el valor final de un isótopo estable es la suma de numerosos procesos metabólicos (Hobson, 1999) puede determinarse un valor promedio del factor de discriminación, por ejemplo, la razón isotópica en el depredador está enriquecida globalmente respecto a su dieta en un 0-1‰, en el caso del $\delta^{13}C$, y alrededor de 3-5‰, en el caso del $\delta^{15}N$ (Hobson et al. 1996; Roth & Hobson, 2000; Kurle & Worthy, 2002).

Entre los elementos de interés biológico más abundantes en la naturaleza se encuentran el carbono (C), el nitrógeno (N) y el oxígeno (O) (Ehleringer & Osmond, 1989). Los valores de $\delta^{13}C$ son buenos indicadores de hábitat, ya que permiten discriminar entre las diferentes fuentes de producción primaria (Fry, 2006). En los ecosistemas acuáticos, la fotosíntesis está condicionada tanto por la disponibilidad de la luz como por la difusión del CO_2. Es así como las algas planctónicas tienen fácil acceso al CO_2 y pueden discriminar contra el ^{13}C, mientras que las algas bentónicas están condicionadas por la cantidad de CO_2 disponible, ya que el acceso al ^{12}C del CO_2 no les resulta fácil. Las diferencias en la accesibilidad al CO_2 entre algas planctónicas y bentónicas se traduce en que las algas bentónicas resultan enriquecidas en ^{13}C respecto a las planctónicas, pudiéndose transmitir estas diferencias a los consumidores (France, 1995), y por lo tanto, generándose el patrón de que las especies de los ecosistemas pelágicos se caracterizarán por valores de ^{13}C inferiores al de los de las especies bentónicas.

Los isótopos de nitrógeno ($^{15}N/^{14}N$) se pueden considerar como indicadores del nivel trófico que ocupan los depredadores (Michener & Schell, 1994). El esquema del metabolismo general del nitrógeno es el siguiente: las proteínas introducidas en el tracto digestivo son transformadas en sus aminoácidos y sufren una desaminación antes de entrar en el sistema de reciclaje metabólico. El nitrógeno así generado es excretado en forma de amonio (preferentemente las formas ligeras ^{14}N) por la mayor parte de los animales acuáticos y ácido úrico o urea en las especies terrestres (Minagawa & Wada, 1984). Como resultado, se va acumulando la forma pesada ^{15}N en los tejidos, y por lo tanto, los valores de $\delta^{15}N$ se van incrementando progresivamente a través de las redes tróficas (DeNiro & Epstein, 1981).



En los ambientes marinos, la mayor parte de la variabilidad de los valores de $\delta^{18}O$ se debe a la evaporación preferencial de agua con el isótopo ligero y la condensación preferencial de las moléculas de agua con el isótopo pesado (Bowen, 2010). Como resultado de esto, existe una claro empobrecimiento en ^{18}O desde los trópicos a los polos (Bowen & Wilkinson, 2002). Estos gradientes latitudinales en los valores de $\delta^{18}O$ de la mayoría de los océanos pueden ser utilizados como marcadores geográficos en diferentes organismos marinos (v.g. Yoshida & Miyazaki, 1991; Jones & Campana, 2009; Soldati *et al.* 2009). Específicamente, en mamíferos marinos los valores de $\delta^{18}O$ de la apatita del hueso reflejan los cambios en los valores de $\delta^{18}O$ de la dieta, y en consecuencia de las masas de agua circundantes, por lo que es posible distinguir los diferentes orígenes geográficos o zonas de alimentación en estos animales (ver tercer capítulo).

El lobo marino sudamericano

El lobo marino sudamericano es un pinnípedo de la familia Otariidae. Desde su descubrimiento, al inicio del siglo XIX, el estatus de su nombre científico ha sido objeto de controversias (ver King 1978; Rodríguez & Bastida 1993; Berta & Churchill 2012) y actualmente dos nombres están en uso: *Otaria flavescens* (Shaw, 1800) y *Otaria byronia* (Blainville, 1820). *Otaria flavescens* fue descrita basado en una cria de 62 cm de largo, con pelaje de color amarillento uniforme colectada en el Estrecho de Magallanes, mientras que el holotipo de *Otaria byronia* proviene de un cráneo colectado en la isla de Tinian (Archipiélago de las Islas Marianas), área que no está dentro del rango de distribución de esta especie. Debido a que la descripción del holotipo y la localidad donde fue hallado el espécimen "flavescens" corresponden a características más comunes con las crías (en proceso de muda) de los lobos marinos sudamericanos (Rodríguez & Bastida 1993), en esta tesis doctoral se utiliza en general el nombre científico *Otaria flavescens*, excepto en el artículo del primer capítulo donde se utilizó *Otaria byronia* a petición de uno de los revisores, el cual sostenía que era más conveniente seguir la nomenclatura científica de la Sociedad de Mamíferos Marinos.

Descripción: También conocido como lobo marino de un pelo, lobo marino común y lobo marino chusco. Es una de las especies de pinnípedos de mayor tamaño; los machos adultos pueden llegar a pesar 350 kilos y medir 2.8 metros de largo; las hembras no superan los 200 Kg. y los 2.2 metros de largo. Las crías al nacer son de 85 cm y con un peso entre 11-15 Kg (Capozzo et al. 1991).

Distribución: El lobo marino sudamericano se distribuye desde la localidad de Zorritos, Perú, a los 4°S sobre el Océano Pacífico, hasta Torres, Brasil, a los 29°S sobre el Océano Atlántico (Sanfelice et al. 1999). En el litoral atlántico argentino se lo encuentra desde Mar del Plata hasta Tierra del Fuego, en más de 85 asentamientos, concentrándose la mayoría en la zona centro-sur de la Patagonia argentina.

Ciclo de vida y comportamiento: El lobo marino sudamericano es una especie con marcado dimorfismo sexual y con un sistema de apareamiento poligínico con defensa de hembras (Campagna & LeBoeuf, 1998). Durante la época de apareamiento (verano austral) los machos arriban a las playas de cría a comienzos de la temporada, compitiendo con otros machos por la posesión de territorios. Las hembras arriban unas semanas después y se agregan formando colonias de unos pocos machos (Campagna & Le Boeuf 1988; Cassini & Vila 1990). El ciclo reproductivo de la especie, al igual que de todos los otáridos, se caracteriza por el apareamiento post-parto y un tiempo de gestación que permanece invariable con duración de 8 meses, lo que unido a la implantación retardada del embrión provoca un parto anual (Boyd et al. 1999).

Durante el período de lactancia, las hembras alternan períodos en los sitios de paridera, alimentando a la cría, con períodos alimentándose en el mar, mientras la cría permanece en la playa (Campagna et al. 2001). Estas últimas permanecen con su madre hasta el año de edad, siendo amamantadas hasta poco más de los 3 meses, edad en la cual aprenden a nadar y comienzan a buscar su alimento (Campagna & Le Boeuf 1988). Las hembras entran en el ciclo reproductivo entre los 4 y los 5 años, mientras que los machos, si bien se desarrollan sexualmente a la misma edad, sólo pueden disputar con otros machos la posesión de hembras y reproducirse efectivamente desde los 9 años de edad (Crespo 1988). Se estima que la longevidad de la especie es de alrededor de 20 años para ambos sexos, tanto en vida libre como en cautiverio.

Dieta: es una especie oportunista que preda sobre una amplia gama de recursos con una tendencia a consumir especies demersales y de hábitos bentónicos, algunas de ellas de importancia comercial.

Estatus y conservación: Desde la segunda mitad del Holoceno los lobos marinos sudamericanos fueron perseguidos y cazados por los seres humanos. Primero los pueblos cazadores–recolectores nativos los utilizaban para su propia economía y alimentación, y luego como consecuencia de la llegada de los europeos las poblaciones disminuyeron dramáticamente a lo largo de toda su área de distribución, ya que fueron explotadas intensamente por su piel y grasa entre los siglos XVIII y XX. En Argentina, a pesar que la explotación cesó en la década del 60 por cuestiones económicas, recién a finales de los 80 algunas poblaciones de la Patagonia comenzaron a mostrar una tasa de incremento poblacional positiva gracias a la implementación de políticas de conservación en la década del 70 (Crespo & Pedraza, 1991). Actualmente la población total estimada corresponde a 275.000 individuos, con alrededor de 95.000-100.000 individuos distribuidos a lo largo del litoral Argentino, donde las principales causas de mortalidad están relacionadas con las interacciones directas e indirectas con las pesquerías (Crespo et al. 1997; Grandi et al. 2012).

OBJETIVOS

“La competencia implica la negación de lo que uno hace,
porque uno hace las cosas en función de lo que hace otro”
Humberto Maturana

El objetivo general de esta tesis doctoral consiste en analizar los efectos de la explotación humana y el desarrollo de la industria pesquera sobre la dieta del lobo marino sudamericano.

La hipótesis inicial es que debido a la amplitud de su nicho ecológico, los lobos marinos sudamericanos se alimentan de varios tipos de presas en función de su abundancia, siendo la competencia intraespecífica el principal determinante de la composición de sus dietas, ya que se alimentan de presas demersales cuándo y donde la población es pequeña y cambian a presas pequeñas pelágicas cuando y donde la población aumenta (Drago et al. 2009b). Si esto fuera cierto, la caza comercial por parte de los colonizadores europeos habría provocado un importante cambio en la posición trófica de esta especie, al reducir de forma notable sus poblaciones. En cambio, la caza aborigen no habría provocado cambios en la dieta del lobo común.

La presente tesis doctoral pretende evaluar estas hipótesis mediante el uso de isótopos estables. Dado que la señal isotópica de base cambia regionalmente en el Atlántico sur, el primer capítulo aborda el estudio de los patrones de dispersión de los lobos marinos sudamericanos mediante el análisis de los isótopos estables de oxígeno en la apatita del hueso, con el fin de determinar si existen diversas unidades de estudio en el Atlántico sur. Debido a que en estos animales la apatita del hueso crece a temperatura constante y el alimento constituye su principal fuente de agua, es probable que los cambios en las razones isotópicas de oxígeno ($\delta^{18}O$) del hueso reflejen los cambios en los valores $\delta^{18}O$ de la dieta, y en consecuencia de la masa de agua circundantes, a pesar de la existencia de factores de confusión como la composición de la dieta, el sexo y el tamaño corporal. En este trabajo utilizamos los valores $\delta^{18}O$ de la apatita en hueso para investigar los patrones de dispersión de machos adultos provenientes de tres regiones del océano atlántico sudoccidental (Brasil, Patagonia y Tierra del Fuego en Argentina), así como también evaluar el grado de la variabilidad de la dispersión en función del sexo y categorías de edad en individuos de la Patagonia.

Una segunda cuestión previa que debe resolverse es la correcta interpretación de los cambios en las razones isotópicas en tejidos inertes, pues estos podrían reflejar no sólo cambios en la dieta, sino además cambios en el factor de discriminación trófica de raíz fisiológica. Esto es así porque en algunos vertebrados se ha detectado cambios cíclicos en las razones isotópicas de los tejidos inertes causados por procesos de ayuno (Beltrán et al 2015), lactancia (Jenkins et al 2001) y gestación (Fuller et al., 2004). Para esto, se propone en el **segundo capítulo** de esta tesis el análisis de isótopos estables de carbono y nitrógeno en vibrisas de lobos marinos sudamericanos adultos en cautiverio, alimentados con una dieta constante, con el fin de evaluar la magnitud de los cambios asociados con el ciclo reproductivo.

Luego, utilizamos esta información para analizar los cambios cíclicos en las razones de los isótopos estables de las vibrisas de individuos de vida libre provenientes de la Patagonia Atlántica.

Una vez aclaradas las dos cuestiones anteriores, ha sido posible evaluar los efectos de los cambios en la demografía del lobo común sobre su dieta. Para ello, en el tercer capítulo se han analizado las razones isotópicas de carbono y nitrógeno en elementos esqueléticos de lobo común sudamericano procedentes de registros zooarqueológicos y de colecciones científicas del siglo XX del norte de Patagonia y de Tierra del Fuego. Como este estudio implica conocer así mismo los cambios en el nivel de la base isótopica del ecosistema a lo largo del Holoceno medio y tardío, se incluye también el análisis de herbívoros (lapas) y suspensívoros (mejillones/cholgas) presentes en el registro arqueológico. Luego, una vez comprendida la posición trófica del lobo marino sudamericano a lo largo del Holoceno medio y tardío, el capítulo cuatro aborda el posible cambio de la dieta del lobo común en Brasil durante las tres últimas décadas del siglo XX, con el fin de evaluar el efecto del desarrollo de la industria pesquera del Río de la Plata y zonas adyacentes. Para eso se analizarán los isótopos estables de carbono y nitrógeno en los huesos del cráneo y dentina de individuos machos provenientes de las colecciones científicas.

PRIMER CAPÍTULO

LA CONECTIVIDAD ENTRE LAS POBLACIONES DE LOBO MARINO SUDAMERICANO

Los isótopos estables de oxígeno revelan los patrones de dispersión de los lobos marinos sudamericanos en el océano Atlántico sudoccidental

RESUMEN. Los isótopos estables de oxígeno han sido ampliamente utilizados para reconstruir paleotemperaturas y para investigar el entorno térmico de peces y moluscos, pero sólo ocasionalmente han sido utilizados como marcadores geográficos en ecosistemas marinos. Debido a que en los mamíferos marinos, la apatita del hueso crece a temperatura constante y el alimento constituye su principal fuente de agua, en particular para los pinnípedos, es probable que los cambios en las razones isotópicas de oxígeno ($\delta^{18}O$) en la apatita de hueso reflejen los cambios en los valores $\delta^{18}O$ de la dieta, y en consecuencia de la masa de agua circundantes, a pesar de la existencia de otros factores como la composición química de la dieta, el sexo y el tamaño corporal que podrían influir. En este trabajo, utilizamos los valores $\delta^{18}O$ de la apatita del hueso para investigar si los machos adultos de los lobos marinos sudamericanos (*Otaria byronia*) provenientes de tres regiones del océano Atlántico sudoccidental (Brasil, Patagonia y Tierra del Fuego), utilizan las mismas masas de agua para alimentarse o si por el contrario existen diferencias en las masas de agua utilizadas. Además, analizamos muestras de ejemplares de diferentes edades y de ambos sexos procedentes de la Patagonia argentina para evaluar si existían diferencias en las masas de agua empleadas en función del sexo y de la etapa de desarrollo. Se observaron diferencias estadísticamente significativas entre los valores de $\delta^{18}O$ de machos adultos de las tres regiones, estando los huesos de los individuos provenientes de Patagonia más enriquecidos en ^{18}O, tal como se esperaba de acuerdo a los valores de $\delta^{18}O$ de agua de mar. Estos resultados revelaron movimientos de dispersión restringidos para los machos adultos entre las tres regiones estudiadas. Por otra parte, machos y hembras adultos de la misma región no difirieron en los valores medios de $\delta^{18}O$ del hueso, indicando el uso de las mismas masa de agua. Además, la variabilidad en los valores de $\delta^{18}O$ en el hueso de individuos jóvenes de ambos sexos fue mucho más amplia que la de los adultos, lo que sugiere la existencia de un fase juvenil de dispersión en ambos sexos, aunque mucho más corta en las hembras que en los machos. Estos resultados confirman la utilidad de los isótopos estables de oxígeno como trazadores de hábitats en mamíferos marinos.

Título original: Stable isotopes of oxygen reveal dispersal patterns of the South American sea lion in the southwestern Atlantic

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Stable isotopes of oxygen reveal dispersal patterns of the South American sea lion in the southwestern Atlantic Ocean

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Keywords
bone; bioapatite; oxygen isotopes; dispersal patterns; habitat; pinnipeds.

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Abstract

Stable isotopes of oxygen have been widely used to reconstruct paleotemperatures and to investigate the thermal environment of fishes and mollusks, but they have only occasionally been used as geographical markers in marine systems. As bone apatite grows at a constant temperature in marine mammals and food is the major source of water for these animals, particularly for pinnipeds, variations in the ratio of stable isotopes of oxygen ($d^{18}O$) of bone apatite will likely reflect changes in the $d^{18}O$ values of diet, and thus of the surrounding water mass, despite the potential confounding role of factors as the proximate composition of diet, sex and body size. Here, we used the $d^{18}O$ values in bone apatite to investigate whether adult males of South American sea lion (*Otaria byronia*), from three regions in southwestern Atlantic Ocean (Brazil, Patagonia and Tierra del Fuego in Argentina), used the same water masses to forage and whether differences exist in the water masses used by sea lions differing according to sex and developmental stage. Statistically significant differences were observed among the $d^{18}O_{bone}$ values of adult males from the three regions, with those from Patagonia more enriched in ^{18}O, as expected from the $d^{18}O_{seawater}$ values. These results revealed restricted dispersal movements of adult males between the three areas. On the other hand, adult males and females from Patagonia did not differ in average $d^{18}O_{bone}$ values, thus indicating the use of foraging grounds within the same water mass. Finally, the variability in the $d^{18}O_{bone}$ values of young of both sexes was much wider than the adults of the same sex from the same region, which suggests the existence of a juvenile dispersal phase in both sexes, although much shorter in females than in males. These results confirm the usefulness of stable isotopes of oxygen as habitats tracers in marine mammals.

Introduction

Recent advances in satellite telemetry have helped to fill the gap in our knowledge of animal movements, but these methods are expensive and tracking is often restricted to a few individuals for relatively short periods (Shillinger *et al.*, 2008). Biogeochemical markers such as stable isotopes lack the detailed resolution of satellite tags, but laboratory analyses are inexpensive and provide information integrated over known and predictable timescales that can be linked directly to geographical regions if the isotopic landscape, or isoscape, has been previously reconstructed (Hobson & Wassenaar, 2008; Graham *et al.*, 2010).

Stable isotopes are known from several chemical elements and the relative abundance of two stable isotopes in a sample is usually expressed as a ratio between the heavy and the light isotope and compared with that ratio in a standard (Hobson & Wassenaar, 2008). Stable isotopes of carbon and nitrogen are among the most widely used biogeochemical markers in ecological studies (Koch, 2008) and maps describing the variability of their stable isotope ratios across entire oceans are available (Graham *et al.*, 2010). However, interpreting changes in $d^{13}C$ and $d^{15}N$ values to track migrations is possible only if no major dietary shifts occur during migrations, which is not necessarily true for opportunistic predators.

Stable isotopes of oxygen have been widely used to reconstruct paleotemperatures (e.g. Schöne *et al.*, 2004) and to investigate the thermal environment of fishes (e.g. Jones & Campana, 2009) and mollusks (e.g. Soldati *et al.*, 2009), because carbonates in biominerals become enriched in ^{18}O as temperature declines (Kim & O'Neil, 1997; Soldati *et al.*, 2009). Nevertheless, most of the current variability in the ratios of stable isotopes of oxygen in the ocean is not linked to thermal gradients, but caused by the preferential evaporation of the light isotope and the preferential condensation of the heavy isotope (Bowen, 2010). Accordingly, a sharp contrast exists between $d^{18}O$ values in freshwater and seawater and well-defined latitudinal gradients of $d^{18}O$ values also exist from mid to high latitudes in most oceans (Bowen, 2010).

Such regularities make stable isotopes of oxygen potential habitats tracers for marine species moving between marine and freshwater ecosystems and for species with distribution ranges spanning over marine regions differing in $d^{18}O$ values. However, stable oxygen isotopes have seldom been used to track the migrations of marine vertebrates (e.g. Yoshida & Miyazaki, 1991; Clementz & Koch, 2001; Coulson *et al.*, 2008; Ramos, González-Solís & Ruiz, 2009). A constant body temperature does not pose any actual limitation to the use of stable isotopes as tracers in marine mammal, as the $d^{18}O$ values of endotherms records dietary information and not body temperature (Koch, Fogel & Tuross, 1994; Bryant & Froelich, 1995; Kohn, 1996; Koch, 2008). Therefore, $d^{18}O$ values can be a useful tracer for investigating marine mammal habitats in areas where well-defined gradients exist.

South American sea lions, *Otaria byronia*, are widely distributed along both coasts of South America (Vaz-Ferreira, 1982). Genetic markers suggest female philopatry and male-mediated gene flow among populations in the southwestern South Atlantic Ocean (Szapkievich *et al*., 1999; Freilich, 2004; Túnez *et al*., 2007, 2010; Artico *et al*., 2010 and Feijoo *et al*., 2011). Tagging confirmed that young females often remain close to their breeding site (Thompson *et al*., 1998; Campagna *et al*., 2001), whereas adult males travel longer distances than females after the breeding season (Vaz-Ferreira, 1982; Rosas *et al*., 1994; Giardino *et al*., 2009). Nevertheless, nothing is known about the actual duration of the juvenile dispersal phase or the actual fidelity of adults to a particular stretch of coastline.

Stable oxygen isotopes could provide an insight into these issues because the $d^{18}O_{seawater}$ values of Patagonia are higher than those from southern Brazil and Tierra del Fuego (Bowen, 2010). Accordingly, the $d^{18}O_{bone}$ values in sea lions from those three regions should not reproduce the local pattern reported above for $d^{18}O_{sewater}$ values if sea lions dispersed over scales of more than 1000 km along the coastline of the southwestern Atlantic Ocean. Furthermore, the $d^{18}O_{bone}$ values in males and females from the same region would differ if males had larger dispersal areas than females along the coastline. Finally, $d^{18}O_{bone}$ values would be similar across age classes of female sea lions from the same region if young females remained close to their natal site, but they would vary across age classes of male sea lions if young males dispersed longer distances from their natal sites before settlement as adults. This paper describes the variability of $d^{18}O_{bone}$ values in South American sea lions to test the three former hypotheses.

Material and methods

Sample collection

A total of 112 bone samples were collected from the skulls of sea lions found dead between 1978 and 2010 in three coastal zones of the southwestern South Atlantic coast of South America. Samples from southern Brazil (from 29°S to 31°S) came from the collection from the Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul at Imbé, Brazil (17 adult males). Samples from central and northern Patagonia coasts (from 40°S to 47°S) (here after Patagonia) came from the collection from the Centro Nacional Patagónico at Puerto Madryn, Argentina (39 males and 39 females covering all the developmental stages). Finally, samples from Tierra del Fuego (from 53°S to 54°S) were obtained from the collection from the Museo Acatushún de Aves y Mamíferos Marinos Australes, near at Ushuaia, Argentina (17 adult males). These three regions differed in salinity levels (Fig. 1a) and $d^{18}O_{seawater}$ gradients (Fig. 1b).

Age, sex and development stage determination

Sea lions obtained from these collections had previously been aged by counting growth layers in the dentine of the canines (after being decalcified in 5% formic or nitric acid and thick sections stained with hematoxylin (Crespo, 1988). Sex was assessed according to secondary sex characteristics at the time of collection and measurements of the skull (Crespo, 1988). The life span of sea lions is around 20 years (Crespo, 1988), with females reaching adulthood at about 4 years of age and males mating for the first time when they are at least 9 years old (Grandi *et al*., 2010). Based on these data, we established four developmental stages; young 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; early adult sexually mature individuals between 7 and 8 years of age for males and between 5 and 7 years of age for females; adult sexually mature individuals between 9 and 12 years of age for males and between 8 and 12 years of age for females and senile sexually mature individuals >13 years old for males and >12 years old for females. The main difference between early adults and adults is that the former can still grow in length, whereas the latter are thought to have ceased length growth.

Sample preparation

Each skull sample used for the isotopic analysis consisted of a fragment of turbinate bone from the nasal cavity, which was

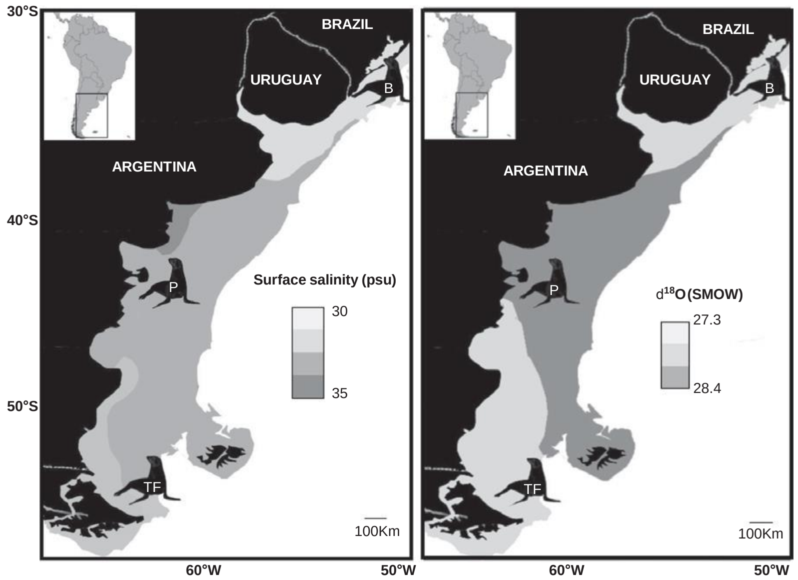

Figure 1 Map of southern South America, showing the study areas (B, southern Brazil; P, central and northern Patagonia; TF, Tierra del Fuego), sea surface salinity [left panel, according to Falabella *et al.* (2009)] and d^{18}O$_{seawater}$ values (right panel, according to Bowen, 2010).

ground with a mortar and pestle. Approximately 15 mg of sample powder were soaked with 30% hydrogen peroxide solution for 24 h, rinsed five times with deionized water, soaked for another 24 h in a solution of acetic acid (1M) buffered to pH~ 4.5 with 1M calcium acetate, rinsed again five times with deionized water and finally dried for 24 h (Koch, Tuross & Fogel, 1997).

Stable isotope analyses

Samples were analyzed for oxygen isotope ratios via a Carbonate Kiel Device III carbonate preparation system (Thermo Electron – Dual Inlet, Thermo Finnigan, Bremen, Germany) linked to a gas source mass spectrometer in the Scientific-Technical Services at the University of Barcelona. Approximately, 1.0 mg of bone powder was dissolved in 100% phosphoric acid at 70°C with concurrent cryogenic trapping of CO_2 and H_2O. The CO_2 was then admitted to the mass spectrometer for analysis. The measured isotope compositions were normalized to the NBS 19 calcite standard, with a value of d^{18}O = -2.20‰ relative to Vienna Pee Dee Belemnite (VPDB). Precision of replicate analyses for d^{18}O was +0.06‰ [standard deviation (*sd*)]. As d^{18}O values in zoology are more commonly presented relative to Vienna Standard Mean Ocean Water (VSMOW), d^{18}O values were converted from VPDB to VSMOW using the formula d^{18}O (VSMOW) = [d^{18}O (VPDB) ¥ 1.03086] + 30.86 (Koch *et al.*, 1997). The stable isotope abundances are expressed in delta (d) notation in parts per thousand (‰), using the formula d^{18}O = [(^{18}O/^{16}O$_{sample}$ --- ^{18}O/^{16}O$_{standard}$ -1) ¥ 1.000], where the standard is VSMOW.

Data analyses

Data are presented as mean + *sd*, unless otherwise stated. Normality was tested with the Lilleford test and homogeneity of variances with the Levene test. The Kruskal–Wallis test was used to compare the d^{8}O$_{bone}$ values of males from southern Brazil, Patagonia and Tierra del Fuego because the three datasets were heteroskedastic (Levene test, $W_{2.48}$ = 7.280, *P* = 0.02). The Kruskal–Wallis test was followed by a *post hoc* nonparametric multiple comparisons test. The Student *t*-test was used to compare d^{18}O$_{bone}$ values of males and females from Patagonia and the Pearson regression analysis was used to test the hypothesis that d^{18}O$_{bone}$ values remained stable after adulthood. Significance was tested at a = 0.05. All statistical analyses were performed with PASW Statistics (Version 17.0 for Windows, SPSS, version 17.0, Spain), except the nonparametric multiple comparison test, performed following Zar (1984).

Results

The bone of male South American sea lions from Patagonia was significantly enriched in ^{18}O when compared with that of males from southern Brazil and Tierra del Fuego (Fig. 2; Kruskal–Wallis test, chi-square = 6.210, d.f. = 2, *P* = 0.045 *n* = 17 for each region). *Post hoc* pairwise comparisons of the mean bone d^{18}O values between regions revealed statistically significant differences between Brazil and Patagonia (*q* = 4.295, *P* = 0.002) and marginally significant differences between Patagonia and Tierra del Fuego (*q* = 3.193, *P* = 0.070), whereas differences between Brazil and Tierra del

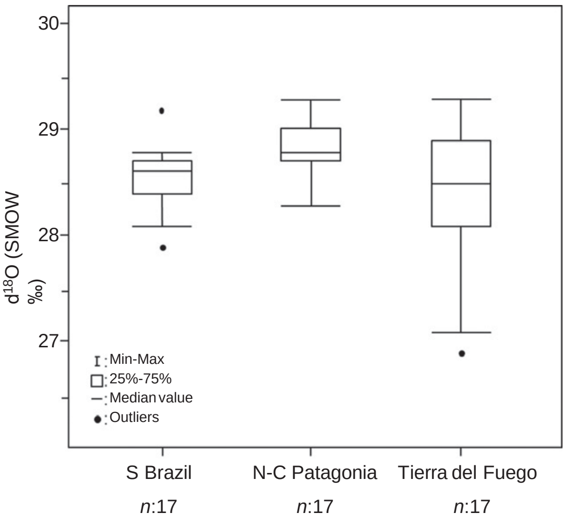

Figure 2 Boxplots of the d^{18}O values in the bone of male South American sea lions, *Otaria byronia*, found dead on beaches in three regions along the coastline of the southwestern South Atlantic Ocean.

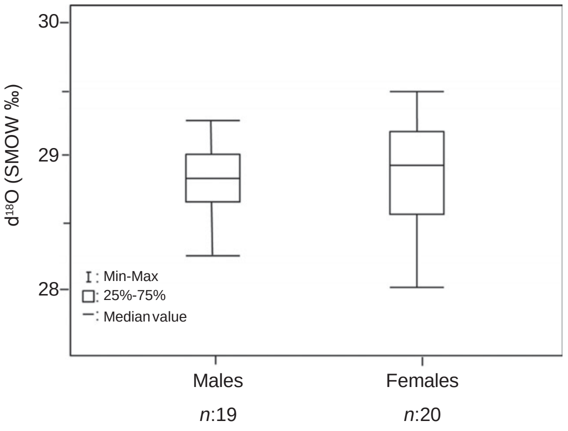

Figure 3 Boxplots of the d^{18}O values in the bone of adult males and females South American sea lions, *Otaria byronia*, found dead in central and northern Patagonia.

Fuego were not statistically significant (q = 0.490, $P > 0.500$). These differences suggested limited exchange of individuals among the three regions.

Adult male and female sea lions from Patagonia did not differ in d^{18}O$_{\text{bone}}$ values (Fig. 3; t = 0.861, d.f. = 37, P = 0.395), indicating that they potentially used the same water masses for foraging. On the other hand, young animals of both sexes exhibited a much larger variability in d^{18}O$_{\text{bone}}$ values in comparison with the adults of the same sex (Fig. 4), as demonstrated by heteroskedasticity both among male (Levene test; males: $W_{3.35}$ = 4.523, P = 0.009) and female developmental stages (Levene test; $W_{3.35}$ = 3.145, P = 0.037). The variability of the d^{18}O$_{\text{bone}}$ values of females decreased sharply at first maturity but remained high in males for several years after sexual maturity. Finally, after first maturity, a statistically significant though moderate decrease in the d^{18}O$_{\text{bone}}$ values occurred in females with age (r^2 = 0.319, P = 0.001), but not in males (P = 0.946).

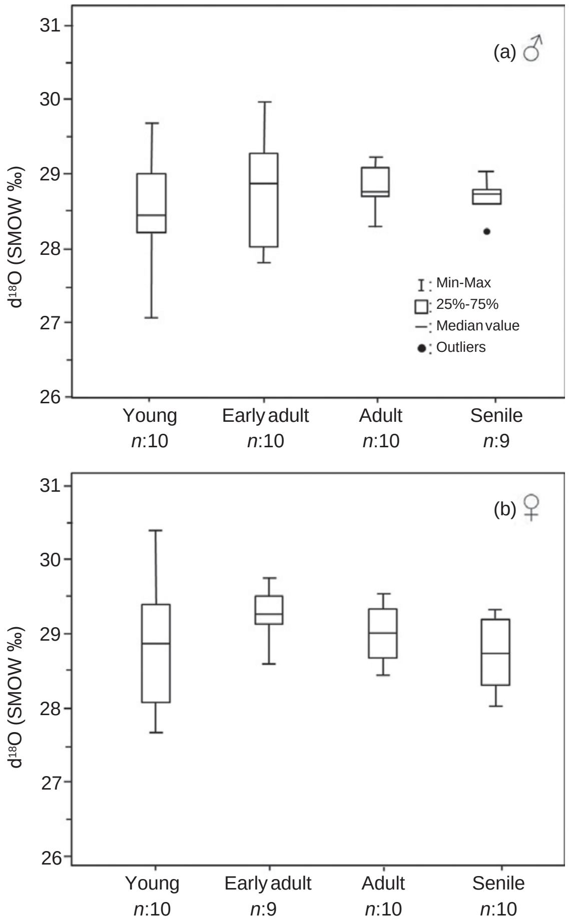

Figure 4 Boxplots of the d^{18}O values in the bone of four developmental stages of South American sea lions, *Otaria byronia*, found dead in central and northern Patagonia. (a) Males; (b) Females.

Discussion

Previous studies using external tags have concluded that adult male South American sea lions may travel longer distances than females after the breeding season (Vaz-Ferreira, 1982; Rosas *et al.*, 1994; Giardino *et al.*, 2009) and may also exhibit a high degree of fidelity to haul-out sites on consecutive nonbreeding seasons (Giardino *et al.*, 2009). Nevertheless, external tags do not last for a long time on sea lions (e.g. Oliveira, 2010) and resightings a few months after tagging are scarce (Giardino *et al*., 2009). Similarly, satellite tags remain attached to sea lions for only a few months and have offered no information about interannual movements (Campagna *et al*., 2001; Riet-Sapriza *et al*., 2012). As a consequence, the actual proportion of adult males moving to distant foraging grounds after the breeding season has remained unknown.

Stable isotope analysis offers an alternative approach, although a number of confounding factors should be considered. Firstly, bone samples come from dead stranded sea lions, which are likely biased for sex ratio and age distribution. However, such biases are not relevant for the hypothesis here to be tested because comparisons among areas were based only on adult males. Furthermore, recent research on dead stranded marine mammals and sea turtles has revealed that decomposition does not significantly modify the stable isotope ratios of soft tissues (Payo Payo *et al*., 2013) and hence is not expected to have any impact on the $d^{18}O$ values of biominerals. The existence of a second set of potential confounding factors is suggested by the water balance models developed for other mammals (Kohn, 1996; O'Grady *et al*., 2012; Podlesak *et al*., 2012). Sea lions obtain the water they need from food (Ortiz, 2001; Berta, Sumich & Kovacs, 2005). South American sea lions consume a diversity of prey species, differing widely in water and fat contents, but not in protein contents (Drago *et al*., 2009b, 2010). Therefore, dietary changes between sexes and age classes (Drago *et al*., 2009a) may result in differences in the relative contribution of metabolic and preformed water to the water supply of sea lions. As the metabolic water generated by food oxidation is ^{18}O-enriched relative to ingested water and preformed water in the diet (Newsome, Clementz & Koch, 2010) and the consumption of fat-rich prey declines with body size in sea lions (Drago *et al*., 2009a), younger developmental stages and females might be more enriched in ^{18}O than older age classes and males, even if they forage in the same water mass.

Reproduction is another potentially confounding factor, not only because lactation results in a high water turnover rate in adult females, but also because male and females differ dramatically in haul-out time and foraging behavior during the breeding season. Adult male sea lions spend as much as 40 days in the beach during the reproductive season (Campagna, 1985) and they fast during that time (Campagna *et al*., 2001). Conversely, females resume feeding as soon as they have been fertilized by males (Campagna & Leboeuf, 1988) and alternate foraging bouts with periods on the beach nursing pups (Campagna *et al*., 2001). Fasting animals rely primarily on the metabolism of fat, and secondarily on protein, to maintain their water balance (Worthy & Lavigne, 1982), and hence fasting for long periods is expected to increase the $d^{18}O$ values of body water. Furthermore, males are exposed to high air temperatures during the breeding season, at least in Uruguay and northern Patagonia, which likely increase water loss through sweating (Khamas *et al*., 2012).

Despite all these potential sources of variability, the absence of statistically significant differences in the average $d^{18}O_{bone}$ values across sexes and developmental stages in the sea lions from Patagonia suggest that these factors considered are actually minor sources of variability. Alternatively, diet might not vary across sexes and developmental stages, but this is unlikely considering the dietary information published to date (Koen-Alonso *et al*., 2000; Drago *et al*., 2009b). Hence, the regional variability in the $d^{18}O_{seawater}$ values stands as the most likely source of variability for the differences observed among males from Brazil, Patagonia and Tierra del Fuego. If so, the results reported here suggest that adult males are quite faithful to a particular coastal region for at least several years because the latitudinal differences observed in the $d^{18}O_{bone}$ values of males were in agreement with the latitudinal patterns of salinity (Guerrero & Piola, 1997) and $d^{18}O_{sea\ water}$ values in the region (Bowen, 2010). In addition, the rate of turnover of oxygen isotopes in hydroxyapatite is assumed to represent several years in large adult mammals (Schwarcz & Schoeninger, 1991; Ambrose & Norr, 1993).

Furthermore, the *sd* of the $d^{18}O_{bone}$ values in males was low in Brazil (+0.32‰) and Patagonia (+0.26‰), but much higher in Tierra del Fuego (+0.67‰). When these figures are compared with the $d^{18}O_{seawater}$ gradients in each region (0.28‰ every 100 km from Rio de Janeiro to Rio de la Plata, 0.11‰ every 100 km from Rio de la Plata to central Patagonia and 0.08‰ every 100 km from central Patagonia to Tierra del Fuego; http://data.giss.nasa.gov/o18data/), males stranding in southern Brazil had foraged along approximately 230 km of coastline, those from Patagonia over 470 km of coastline and those from Tierra del Fuego over 1700 km of coastline (*sd* of $d^{18}O_{bone}$ = +0.67‰). Nevertheless, the high *sd* observed in Tierra del Fuego could be caused by a high degree of individual variability in the use of the relatively diluted channels of the Fuegian Archipelago and the saltier Atlantic waters (Guerrero & Piola, 1997).

Conversely, the absence of differences in the average $d^{18}O_{bone}$ values of adult males and females from Patagonia suggests that they share the same water mass year round, a conclusion that cannot be extrapolated to southern Brazil and Tierra del Fuego because only adult males were analyzed there due to the scarcity of female skulls in the museum collections from those areas. The scarcity of females for southern Brazil is because of the rarity of females in the local haul-out sites (Rosas *et al*., 1994), but this is not true for Tierra del Fuego (Schiavini, Crespo & Szapkievich, 2004).

It should be noted that sharing a water mass does not necessarily means using the same foraging grounds, because the values of $d^{18}O_{seawater}$ vary with latitude but not longitude over the continental shelf of southwestern South America (Bowen, 2010). Hence, animals using foraging grounds at a different distance from the coastline but at the same latitude will not differ in their $d^{18}O_{bone}$ values, which explain why adult male and female from Patagonia have similar $d^{18}O_{bone}$ values although females forage in more coastal areas than males (Campagna *et al*., 2001).

In contrast, the average $d^{18}O_{bone}$ values of young specimens of both sexes collected in northern Patagonia did not differ either from those of the adult and senile individuals, but were more variable. This was also true for early adult males, but not for early adult females. Although the $d^{18}O_{bone}$ values of young specimens integrate the values of hydroxyapatite after weaning with those deposited in uterus and during lactation, the latter signals decays after weaning and vanish totally after several months (Newsome *et al*., 2010).The high variability observed in the $d^{18}O_{bone}$ values of juveniles reveals the presence in the same region of individuals with contrasting foraging histories during the years previous to death which for young specimens could result from differences in age and the persistence of the pre-weaning signals in the youngest animals. This is because suckling mammals are expected to be enriched in the heavier isotopes as compared with their mothers; however, suckling signal decays after weaning (Newsome *et al*., 2010). Although this pattern is well established for nitrogen (Newsome *et al*., 2010) and some of the young individuals analyzed here were young enough to exhibit traces of the suckling signal in their nitrogen stable isotope ratios (Drago *et al*., 2009a), experimental evidence supporting the existence of a suckling signal for oxygen stable isotope ratios in bone is ambiguous (Williams, White & Longstaffe, 2005; Kirsanow & Tuross, 2011). However, no trace of the suckling signal is expected to remain in early adult males (Drago *et al*., 2009a) and hence the high *sd* observed in that group, when compared with that of older males, should have a different explanation.

Actually, the range of $d^{18}O_{bone}$ values observed in young specimens of both sexes and in early adult males greatly overlapped with the range of $d^{18}O_{bone}$ values reported for adult males across the study area, which suggests that young specimens of both sexes disperse before settlement along most of the range of the species in the southwestern Atlantic Ocean, from Uruguay to Tierra del Fuego. This is in sharp contrast with the dispersal pattern of young Steller sea lions (*Eumetopias jubatus*) in the North Pacific, where only males conducted long distance (>500 km) movements, although the range of round trip distance of juveniles increases with age in both sexes (Raum-Suryan *et al*., 2004).

Genetic markers suggested philopatry for females, but not for males, which will disperse over much larger ranges and will be responsible for gene flow (Szapkievich *et al*., 1999; Freilich, 2004; Túnez *et al*., 2007, 2010; Artico *et al*., 2010 and Feijoo *et al*., 2011). The sudden decreases in the variability of the $d^{18}O_{bone}$ values of females observed after adulthood cannot be explained by bone remodeling (Schwarcz & Schoeninger, 1991; Ambrose & Norr, 1993) and suggest that females come back to their natal regions for settlement. Conversely, the *sd* of the $d^{18}O_{bone}$ values of males decreases more slowly as they grow older at a rate consistent with the expected apatite turnover, suggesting that males do not necessarily come back to their natal areas for settlement. Comparing the *sd* of $d^{18}O_{bone}$ values observed in young male sea lions from Patagonia (+0.81‰) and the gradient of $d^{18}O_{seawater}$ values above reported for that region suggests that young males originated from a 2000 km stretch of coastline. Nevertheless, the duration of the juvenile dispersal phase is much longer in males (8 years) than in females (3 years), as suggested by the sudden decline of the variability of the $d^{18}O_{bone}$ after adulthood in females but not in males. Therefore, the overall evidence indicates that both males and females may disperse over long distances as juveniles, but females do not settle far from their natal region although males can, as previously reported for other pinnipeds (e.g. Burg, Trites & Smith, 1999; Hoffman *et al*., 2006; González-Suárez *et al*., 2009).

In conclusion, the results are consistent with information from genetic markers indicating population isolation by distance and male-mediated gene flow, but suggest that once they become reproductively active, early adult males settling far away from their natal rookeries are the ones responsible for gene flow. Furthermore, these results demonstrate that stable isotopes of oxygen represent a useful and inexpensive approach to the study habitat use and dispersal patterns in marine mammals, and particularly highlight the importance of the bone material deposited in museums and other scientific collections as a source of samples.

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SEGUNDO CAPÍTULO

CAMBIOS EN LAS RAZONES ISOTÓPICAS DE TEJIDOS INERTES

Las razones isotópicas en tejidos metabólicamente inertes: más que un cambio de dieta

RESUMEN. Las razones isotópicas en muestras de tejidos metabólicamente inertes, se utilizan con frecuencia para inferir cambios en las dietas de animales salvajes, basándose en la suposición de que la variación de los valores de $\delta^{13}C$ y $\delta^{15}N$ se debe únicamente a las variaciones en la dieta. Sin embargo, el ayuno, la gestación y la lactancia también pueden influir sobre los valores de las razones de isótopos estables en varios tejidos y, por lo tanto, estos procesos pueden ser utilizados para explicar parte de la variabilidad observada de las razones isotópicas en tejidos metabólicamente inertes. Aquí, hemos analizado los valores de $\delta^{13}C$ y $\delta^{15}N$ en vibrisas de lobos marinos sudamericanos adultos (*Otaria flavescens*) en cautiverio, alimentados con una dieta constante, con el objetivo de evaluar la magnitud de los cambios asociados con el ciclo reproductivo, y luego utilizamos esta información para analizar, en la misma especie, los cambios de las razones isotópicas en las vibrisas de individuos de vida libre, provenientes de la Patagonia Atlántica. Los resultados aquí presentados muestran claramente que los valores de $\delta^{13}C$ y $\delta^{15}N$ en los machos en cautiverio cayeron durante la época de celo, mientras que en las hembras, los valores de $\delta^{13}C$ se incrementaron durante el período central de la gestación, y los valores de $\delta^{15}N$ se incrementaron durante la lactancia. Por otra parte, los valores de $\delta^{13}C$ y $\delta^{15}N$ en los individuos adultos de vida libre también fluctuaron periódicamente, pero la amplitud de la oscilación fue generalmente mayor que la de los individuos en cautiverio. Esto indica que los cambios de dieta tienen un importante papel en las fluctuaciones de las razones isotópicas de los individuos de vida libre, excepto para los machos, en los que las fuertes caídas en los valores de $\delta^{13}C$ y $\delta^{15}N$ resultan del prolongado período de ayuno durante la temporada de apareamiento y no a causa de un cambio de dieta.

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Stable isotope values in metabolically inert tissues: more than just dietary changes

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Abstract

Stable isotope values in metabolically inert tissues are often used to infer temporal dietary changes in wildlife, assuming that variation in $\delta^{13}C$ and $\delta^{15}N$ values are due solely to variations in diet. However, fasting, pregnancy and lactation may influence the stable isotope values of several tissues and hence these processes may contribute to explain some of the variability observed in the stable isotope values of metabolically inert tissues. Here, we have analyzed the $\delta^{13}C$ and $\delta^{15}N$ values in the vibrissa of captive adult breeding South American sea lions (*Otaria flavescens*) fed a constant diet to assess the magnitude of the changes associated to the reproductive cycle and then we use this information to analyze the change in stable isotope values along the vibrissa from wild individuals of the same species from Atlantic Patagonia. The results reported here clearly show that the $\delta^{13}C$ and $\delta^{15}N$ of captive males dropped during the rutting season, the $\delta^{13}C$values of females increased during the central part of pregnancy and the $\delta^{15}N$ values of females increased during lactation. The $\delta^{13}C$ and $\delta^{15}N$ values of adult wild specimens also fluctuated periodically, but the amplitude of the oscillation was usually larger than in captive animals. This indicates that diet shifts have a major role on the fluctuations of stable isotope values of wild individuals, except in males, where major drops in $\delta^{13}C$ and $\delta^{15}N$ values result from fasting during the mating season and not because of a diet shift.

Introduction

Stable isotope analysis has become a standard technique in ecological studies during the past two decades (Rubenstein and Hobson 2004; West et al. 2006: Martínez del Rio et al. 2009). The basic assumption of the method is that stable isotope ratios in animal tissues integrate those in diet, plus a trophic discrimination factor that is tissue, diet and taxa specific (Caut et al. 2009). The time window integrated by each tissue depends on its turnover rate and ranges from a few days to several years. However, metabolically inert tissues represent a consumer's diet at the time of deposition and hence these tissues can be used as a timeline of the consumer's isotopic history. Taking advantage of this, changes in stable isotope ratios across dentine growth layers have been used to reconstruct ontogenetic dietary changes in toothed marine mammals (Hobson and Sease, 1998; Newsome et al. 2006, 2009a; Mendes et al. 2007; Knoff et al. 2008; Borrell et al., 2013), changes across carapace scutes to study the ontogenetic dietary shift of sea turtles (Reich et al. 2007; Cardona et al. 2009) and those along baleen plates to study the migratory movements of whales (Schell et al. 1989; Hobson and Schell 1998 Caraveo-Patiño and Soto 2005; Summers et al. 2006; Newland et al. 2011; Aguilar et al. 2014). Furthermore, laboratory experiments have reported negligible variation in $\delta^{13}C$ and $\delta^{15}N$ values along the vibrissa of captive otariids and phocids kept on a constant diet (Hobson et al. 1996; Hirons et al. 2001), hence suggesting that any departure from baseline variation along the vibrissa shafts of wild marine mammals could be interpreted as evidence for dietary change. This approach has been used to study the migratory movements (Cherel et al. 2009; Kernaléguen et al. 2012, 2015), ontogenetic dietary shifts (Kernaléguen et al. 2012; Vales et al. 2015) and individual foraging specialization (Lowther and Goldsworthy, 2011; Kernaléguen et al. 2015; Kernáleguen et al. in press; Baylis et al. 2015) of eared seals. However, the proper interpretation of changes in stable isotope ratios along inert tissues requires accurate information about their actual deposition/growth rate, which is not always available. For instance, the vibrissae of otariids exhibit consistent growth and multiyear retention (Hirons et al., 2001; Cherel et al., 2009) and as a consequence, they preserve a continuous record of shifts in stable isotope values (Hirons et al. 2001; Cherel et al., 2009; Kernaléguen et al. 2012, 2015; Vales et al. 2015; Baylis et al. 2015). Contrary to this, most phocids (Pinnipedia: Phocidae) exhibit annual shedding patterns with decreasing growth rate as vibrissa increase in length and hence interpretation of changes in stable isotope ratios is extremely complex (Hirons et al., 2001; Greaves et al., 2004; Zhao & Schell, 2004; Newland et al., 2011; Beltran et al. 2015).

Metabolic induced changes in the trophic discrimination factor is a second confounding factor often neglected when interpreting temporal changes in the stable isotope ratios recorded in inert tissues. Fasting has strong influence on the stable isotope ratios of some vertebrates (Hobson et al. 1993; Polischuk et al. 2001; Fuller et al. 2005), but not in others (Kempster et al. 2007; Gómez-Campos et al. 2011; Aguilar et al. 2104) and hence some of the cyclical changes in the stable isotope values reported from the inert tissues of free-ranging mammals have been suggested to be caused by restricted food availability (Beltran et al. 2015; Matthews and Ferguson 2015; Vales et al. 2105). On the other hand, the stable isotope values of milk varies with that of the mother's tissues (Jenkins et al. 2001; Habran et al. 2010; Cherel et al. 2015; Borrell et al., 2016) and hence lactation might result into major changes in the stable isotope values of lactating females, although to our knowledge no experimental evidence exists that proves this process. Even pregnancy has been reported to modify nitrogen balance and hence trophic discrimination factors (Fuller et al. 2004). If so, the interpretation of the stable isotope record in inert tissues would be more complex than usually assumed.

In this paper, we have analyzed the stable isotopes of carbon and nitrogen in the vibrissa of captive adult breeding South American sea lions (*Otaria flavescens*) fed a constant diet to assess the magnitude of the changes associated to the reproductive cycle and then we used this information to analyze the cyclical change in stable isotope values along the vibrissa from wild individuals of the same species from Atlantic Patagonia. The final goal of the study is to test two hypotheses: that males disperse over broad areas after the breeding season (Giardino et al. in press, but see Zenteno et al. 2013) and that females increase the consumption of offshore prey as pups grow (Drago et al. 2010).

Material and methods

Captive animals

Vibrissae were collected in February 22nd, 2012 from one adult male sea lion (Alvin) and one adult female (Demi) from the reproductive group kept in captivity at the Oceanogràfic (Valencia, Spain). The male was 8 years old and had been in the rutting state during the three previous boreal summers, during which he reduced dramatically its food consumption from June to September. The female was 11 years old, but had given birth only twice: July 6th, 2009 and July 16th, 2010. Both individuals had been held on a constant diet of thawed herring *Clupea harengus* and capelin *Mallotus villosus* for at least 3 years prior to isotopic analysis. Vibrissae were cut at their base, so the intradermal section of the vibrissa was unsampled. Fish sample (n=5 for each species) were also collected for stable isotopic analysis at the beginning and the end of the experiment.

Wild animals

Vibrissae from 5 adult male and 5 adult female sea lions were collected from carcasses stranded in northern and central Patagonia from 2006 to 2011 (Table 1). Samples include the follicle, so the whole vibrissa was available for analysis. The age of the sampled individuals had previously been assessed by counting growth layers in the dentine of their canines (Crespo, 1988; Crespo *et al.*, 1994). No manipulation of live animals occurred during this study, as sea lions were found dead in beaches. Stable isotope ratios in potential prey were taken from previous studies (Saporiti et al. 2015).

Stable isotope analysis

Vibrissae were stored at ambient temperature until processing. Once in the laboratory, vibrissae were washed in a chloroform/methanol (2:1) solution and cleaned in distilled water for 5 min in an ultrasonic bath to eliminate any surface contaminants (Newsome et al. 2010). The entire vibrissae, from the most recent part synthesized at the base to the tip, were measured, dried in a stove at 60ºC for 36h and cut into 3mm long consecutive sections starting from the proximal end (Cherel et al. 2009). The length of the intradermal vibrissal length was assumed to be 16.0 ± 3.9 mm, as reported by Sadou et al. (2014) for California sea lions (*Zalophus californianus*).

White muscle from Argentine hake (*Merluccius hubbsi*) and mantle muscle from red octopus (*Enteroctopus megalocyathus*), two major prey of southern sea lions in Patagonia, were ground to a fine powder with a mortar and pestle after being dried at 60 ºC in a stove. Lipids can bias the analyses by decreasing $\delta^{13}C$ levels (DeNiro and Epstein 1977), so they were removed from the samples using a sequential soak in a chloroform: methanol (2:1) solution and shaken with a rotator to accelerate the lipid extraction.

Approximately 0.3 mg of vibrissa and 0.3 mg of muscle from fish and octopus 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 the Centres Científics i Tecnològics of the Universitat de Barcelona. The abundances of stable isotopes, expressed in delta (δ) notation, were calculated as the relative variations of stable isotope ratios expressed as permil (‰) deviations from predefined international standards as:

$$\delta X = (R\ \text{sample}/\ R\ \text{standard}) - 1$$

where X is ^{13}C or ^{15}N, and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the sample and standard, respectively. The $\delta^{13}C$ standard was Vienna PeeDee Belemnite (VPDB) calcium carbonate, and the $\delta^{15}N$ standard was atmospheric nitrogen (N_2). International standards (ammonium sulphate, potassium nitrate, glutamic acid for $\delta^{15}N$ and polyethylene, sucrose and glutamic acid for $\delta^{13}C$) were inserted after every 12 samples to calibrate the system and compensate for any drift over time. Precision and accuracy for both $\delta^{13}C$ and $\delta^{15}N$ measurements were 0.1 ‰ and 0.3‰, respectively.

Data analysis

Data are always shown as means ± standard deviation (SD), and significance was established at the 0.05 level. As the assumptions of normality (using Kolmogorov-Smirnov-test) and homocedasticity (using Levene's test) were met, parametric approaches (Pearson correlation and *t* test) were used to compare population averages. Fourier analysis was used to determine existence of significant periodical oscillations.

Results

Captive animals

According to the daily record of food consumption, Alvin's diet included 83.3% herring and 16.7% capelin, whereas Demi's diet included 68.75% herring and 31.25% capelin. The $\delta^{13}C$ and $\delta^{15}N$ values of Alvin's diet were -18.8 ‰ and 12.1 ‰ respectively and those of Demi's diet were -18.9 ‰ and 11.9 ‰. This resulted in an average prey to predator trophic discrimination factor of 2.5 ± 0.4 ‰ for $\delta^{13}C$ and 4.0 ± 0.1 ‰ for $\delta^{15}N$.

Stable isotope ratios varied largely along the vibrissae of both specimens (Figure 1), although they were offered the same food during the whole study. $\delta^{13}C$ and $\delta^{15}N$ values did not differ significantly between individuals (Table 1; t test, p>0.05 for both). Fourier analysis revealed the existence of two overlapping periodical oscillations both for $\delta^{15}N$ and $\delta^{13}C$ in both Alvin and Demi (Tale 1), although they differed in their temporal patterns (Figure 1).

As vibrissae were collected simultaneously from Alvin and Demi and periodical oscillations for $\delta^{15}N$ values had similar periods in both specimens, growth rate was considered to be similar and hence their temporal profiles were compared directly. The most obvious change along Alvin's vibrissae was a simultaneous drop in $\delta^{13}C$ and $\delta^{15}N$ at 1.5 cm, 8.1 cm and 13.8 cm from the base, whereas the most obvious change along Demi's vibrissa was a peak in $\delta^{13}C$ at 4.0 cm, 10.6

cm and 16.9 cm from the base (Figure 1). The simultaneous change in δ^{13}C and δ^{15}N values along Alvin's vibrissae resulted in a significant correlation (Table 1). Interestingly, peaks in the δ^{13}C values of Demi's preceded drops in the δ^{13}C and δ^{15}N values of Alvin by 2.7 ± 0.3 cm. Assuming an annual periodicity for those oscillations, the average vibrissae growth rate in Alvin and Demi was 0.17 mm day^{-1}. Accordingly, the peaks in the δ^{13}C values of Demi predated in 162.7 ± 20.9 days the drops in Alvin's δ^{13}C and δ^{15}N values. The length of the intradermal vibrissae was assumed to be 16.0 mm and it was supposed to have grown during the 96 days prior to sampling. If so, the first 3 mm section of the Alvin and Demi's vibrissa integrated their diets from 97 to 115 days before the sampling date (February 22nd, 2012), i.e. from October 30th to November 17th, 2011. Accordingly, the first drop in the δ^{13}C and δ^{15}N values of Alvin corresponded to the period August 19th -September 6th, 2011 and the two subsequent drops to late summer 2010 and late summer 2009, assuming annual periodicity. Interestingly, Alvin had been in the rutting state from June to September every year from 2009 to 2011 and its daily rate of food ingestion decreased dramatically at that time.

Specimen	Sex	Age	Length (cm)	δ^{13}C (‰)	δ^{15}N (‰)	Correlation between δ^{13}C and δ^{15}N		Fourier analysis Period of oscillation (3 mm sections)	
						r	P	δ^{13}C	δ^{15}N
				Captive					
Alvin	♂	8	14.4	-16.0 ±0.2	16.0 ±0.5	0.744	<0.001	25/13	25/8
Demi	♀	11	18.3	-16.2 ±0.3	16.0 ± 0.2	0.129	0.312	25/13	17/9
				Wild					
OF 817	♂	9	15.8	-12.5 ± 0.4	22.5 ± 0.9	0.792	<0.001	20/9	13/9
OF 1017	♂	10	12.5	-11.7 ± 0.3	22.5 ± 0.3	0.635	<0.001	25/14	25/14
OF 1117	♂	9	7.1	-13.0 ± 0.5	20.3 ± 0.6	0.681	<0.001	13/5	13/6
OF 23	♂	Adult	10.8	-13.6 ± 0.5	20.1 ± 0.4	0.439	0.007	20/9	20/9
OF 1100	♂	4+	14.2	-13.0 ± 0.4	20.0 ± 0.4	0.319	0.029	17/4	17/8
OF 1081	♀	Adult	7.1	-13.3 ± 0.7	20.7 ± 0.8	0.756	<0.001	11/3	25/13
OF 1071	♀	20	9.4	-13.6 ± 0.3	20.4 ± 0.6	0.784	<0.001	17/8	14/8
OF 1128	♀	14+	11.6	-13.1 ± 0.6	20.8 ± 0.5	0.693	<0.001	13/6	13/6
OF 1068	♀	6	12.8	-13.7 ± 0.4	21.2 ± 0.6	0.360	0.018	14/8	11/6
OF 1138	♀	6+	10.2	-13.3 ± 0.6	20.5 ± 0.7	0.434	0.010	17/7	25/11

Table 1. Summary statistics of the vibrissae analyzed.

Consequently, the periodical drops in δ^{13}C and δ^{15}N likely corresponded to the last part of the rutting period and resulted from reduced food consumption. Conversely, Demi's δ^{13}C values peaked during May and June 2009, 2010 and 201, although the 2009 and 2010 peaks were higher and lasted for longer. It is worth noting that Demi gave birth in 2009 and 2010. She was pregnant but did not gave birth in 2011, so the height and the duration of the peak is likely related to the development of the fetus. Finally, it is also worth noting that Demi's δ^{15}N values initially decreased after parturition to increase steadily during the following lactation period. In summary, δ^{13}C values peaked during the central part of pregnancy and δ^{15}N values peaked during lactation. This explains why δ^{13}C and δ^{15}N values were uncorrelated in Demi's vibrissa (Table 1).

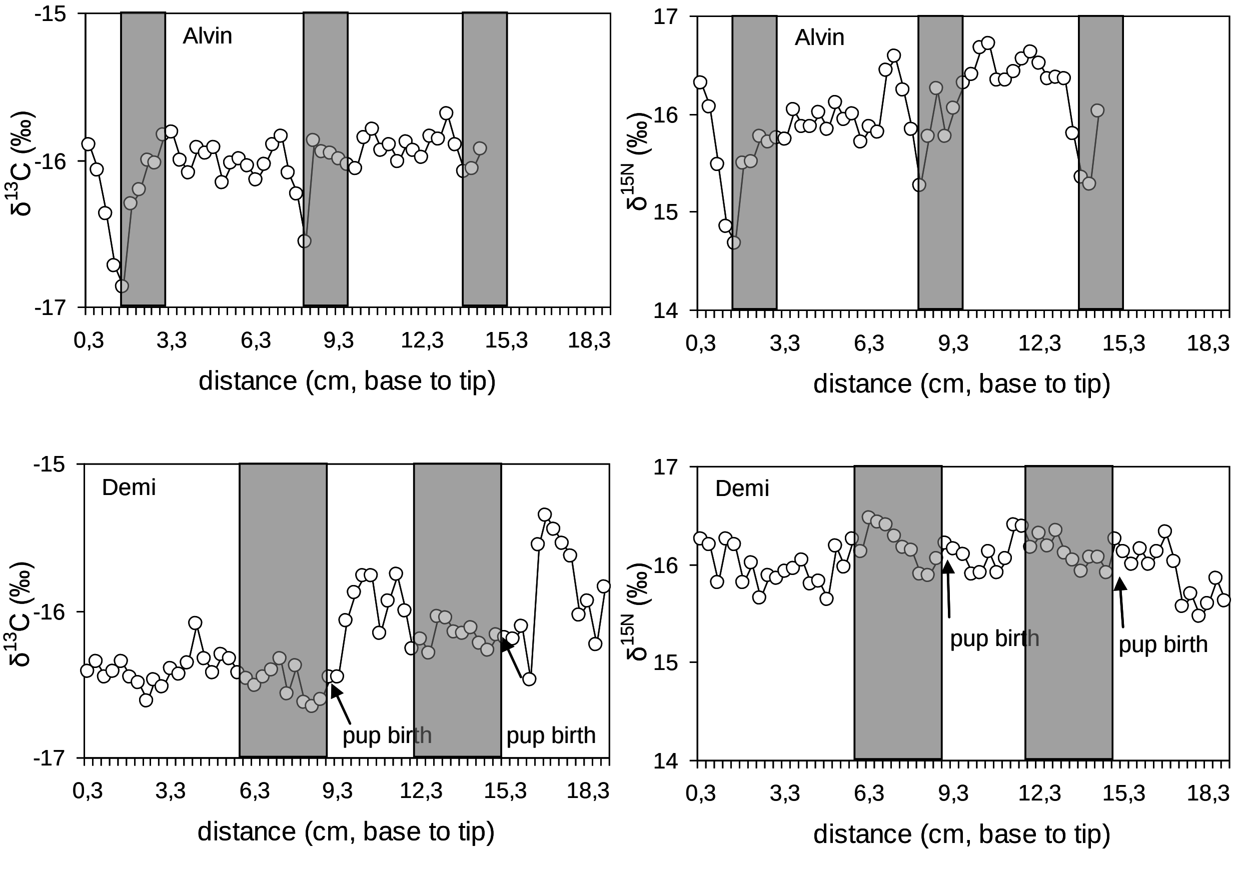

Figure 1. Profiles of δ^{13}C and δ^{15}N along the vibrissa of an adult male (Alvin, top panels) and an adult female (Demi, bottom panels) of South American sea lion kept in captivity. Shadow sections show the rutting period of Alvin in 2009, 2010 and 2011 (June to September) and the lactation period of Demi in 2009 and 2010 (July to January).

Wild animals

The stable isotope values of vibrissae of the five adult males studied varied cyclically (Figures 2) and there was a significant correlation between $\delta^{13}C$ and $\delta^{15}N$, although it was weak in the youngest specimens (Table 1). The average period of the $\delta^{13}C$ cycles in males was 19.0 ± 3.4 sections and that of the $\delta^{15}N$ cycles was 17.6 ± 4.5 sections (Table 1), which were significantly shorter than the cycles observed in captivity ($\delta^{13}C$: $t = 3.038$, $df = 4$, $p = 0.038$; $\delta^{15}N$: $t = 3.258$, $df = 4$, $p = 0.031$). On the other hand, the amplitude of the stable isotope variation was larger than in captive individuals for the $\delta^{13}C$ values (average: 1.6 ± 0.23 ‰; $t = 3.302$, $df = 4$, $p = 0.030$) but not for those of $\delta^{15}N$ (average: 1.9 ± 0.9 ‰ $t = 2.000$, $df = 4$, $p = 0.116$). Physiological changes associated to the breeding cycle were the most likely reason for the periodical drops in $\delta^{15}N$, whereas dietary changes might be also involved in the periodical changes in $\delta^{13}C$, at least in some individuals. For instance, the oldest sections of male OF 1117 showed a drop in $\delta^{13}C$ and $\delta^{15}N$ values similar to that observed in Alvin at the end of the rutting period, but neither $\delta^{13}C$ nor $\delta^{15}N$ rose again to original levels. Conversely, the values of both elements recovered after a second drop which was observed 17 sections after the first one and likely indicative of a second fasting period. A possible explanation of that pattern is that after the first breeding season recorded in the vibrissa, male OF 1117 shifted to a more pelagic diet. Likewise, the $\delta^{13}C$ and $\delta^{15}N$ values of male OF 23 revealed two major drops compatible with fasting during the breeding season. However, after the most recent drop, the $\delta^{15}N$ values recovered quickly to the previous level, as expected, but the values of $\delta^{13}C$ fluctuated widely and frequently, probably indicating prey shifts. Finally, the $\delta^{15}N$ values of male OF 1100 exhibited three well defined oscillations, but only the two most recent ones were associated to synchronous oscillations of the $\delta^{13}C$ values. Conversely, the $\delta^{13}C$ values fluctuated largely along the oldest part of the vibrissa, probably indicating prey shifts.

The $\delta^{13}C$ and the $\delta^{15}N$ values of wild females also varied cyclically (Figure 3). The average period of $\delta^{13}C$ cycles was 14.4 ± 2.6 sections and the average period of $\delta^{15}N$ cycles was 17.6 ± 6.8 sections. These figures were significantly shorter than the cycles observed in captivity for $\delta^{13}C$ ($t = 9.089$, $df = 4$, $p = 0.001$) but not for $\delta^{15}N$ ($t = 0.196$, $df = 4$, $p = 0.854$). The amplitude of the stable isotope variation was larger than in captive individuals for $\delta^{15}N$ (average: 2.4 ± 0.3 ‰ $t = 11.068$, $df = 4$, $p = <0.001$) and differences were in the verge of similarity for the $\delta^{13}C$ values (average: 2.1 ± 0.4 ‰; $t = 2.361$, $df = 4$, $p = 0.078$). Accordingly, physiological changes associated to the breeding cycle could not explain all the variability observed and dietary changes were likely involved. This probably explains why the $\delta^{15}N$ values of the wild females cycled in phase with their $\delta^{13}C$ values and hence the $\delta^{13}C$ and $\delta^{15}N$ values were positively correlated, although correlation was weak in the youngest specimens (Table 1).

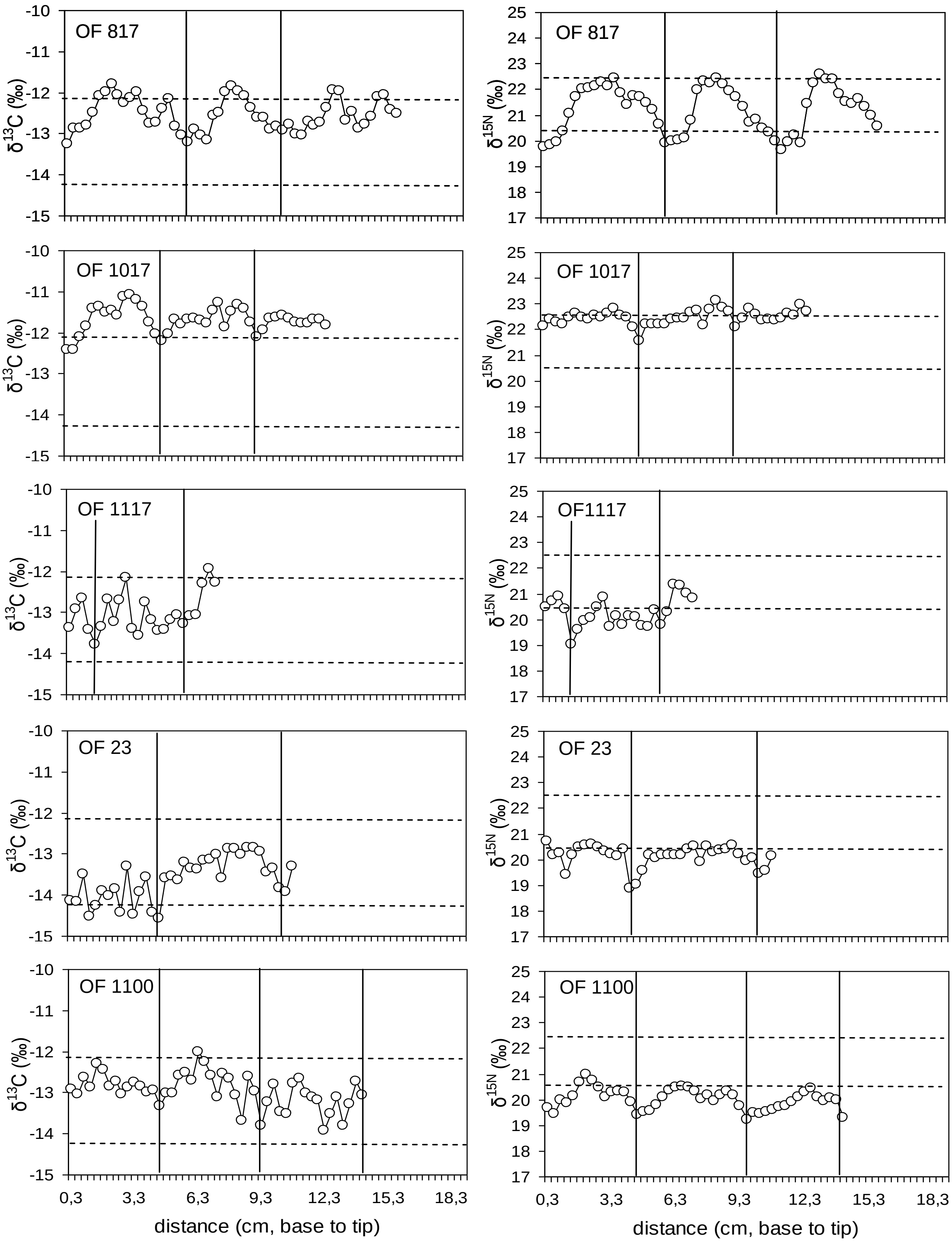

Figure 2. Profiles of δ13C and δ15N along the vibrissae of five wild adult male South America sea lions. Horizontal dashed lines show the expected stable isotope ratios for octopus (top) and hake only diets (bottom), after accounting for the trophic discrimination factor. Vertical lines show the limits of annual cycles, set at the recovery of the basal stable isotope ratio after the drop associated to the rutting period.

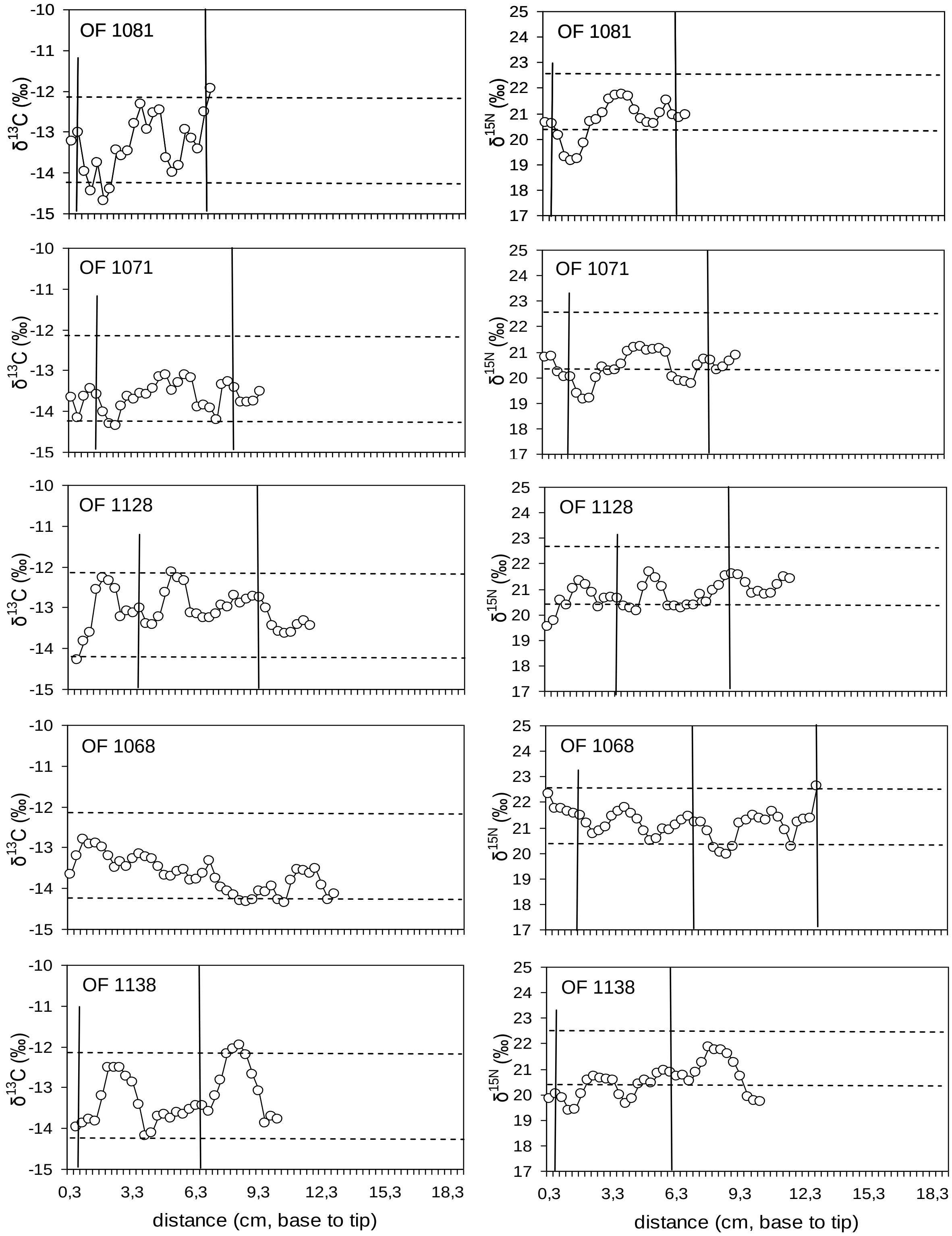

Figure 3. Profiles of $\delta^{13}C$ and $\delta^{15}N$ along the vibrissae of five wild adult female sea lions. Horizontal dashed lines show the expected stable isotope ratios for octopus (top) and hake only diets (bottom), after accounting for the trophic discrimination factor. Vertical lines show the limits of annual cycles, set at the recovery of the basal stable isotope ratio after the drop associated to weaning.

Discussion

The results reported here clearly show that the $\delta^{13}C$ and $\delta^{15}N$ of adult captive South American sea lions kept on a constant diet exhibited broad variations likely related to the physiological changes associated to the breeding cycle. More precisely, the $\delta^{13}C$ and $\delta^{15}N$ values of males dropped during the rutting season, the $\delta^{13}C$values of females increased during the central part of pregnancy and the $\delta^{15}N$ values of females increased during lactation. The $\delta^{13}C$ and $\delta^{15}N$ values of adult wild specimens also fluctuated periodically, but the amplitude of the oscillation was usually larger in wild animals, thus highlighting the role that diet shifts have on stable isotope values.

Previous research revealed little variation in the $\delta^{13}C$ and $\delta^{15}N$ values along the length of vibrissa of adult captive seals and sea lions kept on constant diets and any fluctuation was attributed to the variance in isotopic composition of the diet (Hobson et al. 1996; Hirons et al. 2001). Accordingly, cyclical fluctuations observed in wild otariids have been systematically interpreted as evidence of periodical shifts between isotopically dissimilar prey or foraging grounds (Hirons et al. 2001; Cherel et al. 2009; Kernálegen et al. 2012, 2015, 2015; Vales et al. 2015; Baylis et al. 2015). However, the results reported here exhibited strong and periodical oscillations of the $\delta^{13}C$ and $\delta^{15}N$ values of reproductively active, adult captive South American sea lions kept on a constant diet and hence this is of relevance when interpreting seasonal patterns of variability in wild otariids.

Wild male South American sea lions fast for as long as 40 days during the breeding season on the austral summer (Campagna, 1985) and the captive male studied here also reduced food intake dramatically during the boreal summer months. Research on birds suggests that fasting results into no change in $\delta^{13}C$ values but into an increase in $\delta^{15}N$ values due to protein mobilization and subsequent preferential excretion of ^{14}N (Hobson et al. 1993). However, further research has revealed that, in marine mammals, fasting results in no change in stable isotope ratios (Gómez-Campos et al. 2011) or in a decrease in both $\delta^{13}C$ and $\delta^{15}N$ (Polischuk et al. 2001; Aguilar et al. 2104; Matthews and Ferguson, 2015; Vales et al. 2105). The reasons for these contrasting results are not fully understood, but the high volume of fat reserves in marine mammals that mitigate the need for protein mobilization (Gómez-Campos et al. 2011) and the possible capacity to recycle nitrogen to avoid osmotic unbalance during fasting have been invoked (Aguilar et al. 2014). Whatever the actual physiological explanation, the cyclical drops of $\delta^{13}C$ and $\delta^{15}N$ observed in the vibrissa of the captive male here studied, together with the fact that the lowest values were observed at the end of the fasting period, strongly support the hypothesis that fasting indeed causes dramatic drops in the δ^{13}C and δ^{15}N values of marine mammals.

Opposite to males, female otariids alternate fasting and feeding bouts during the period of pup rearing and are expected to rely primarily on diet to produce milk (Berta and Sumich, 1999). In the otariids studied to date, milk protein is consistently depleted in both ^{13}C and ^{15}N as compared to the blood of lactating females (Cherel et al. 2015) and this is also true for the whole milk of a diversity of other income breeders (Jenkins et al. 2001). This suggests that lactation should result into the enrichment of female's tissues in ^{13}C and ^{15}N as lactation progresses. Enrichment in ^{13}C is expected to be particularly intense in lactating female otariids, as they produce milk with high fat contents and thus depleted in ^{13}C when compared with female tissues (Bert and Sumich, 1999; Newsome et al. 2006). The situation is different in female capital breeders which fast through lactation (Polischuck et al. 2001; Ducatez et al. 2008; Habran et al. 2010; Borrell et al. 2016) probably because fasting and lactogenesis have opposing effects of the stable isotope ratios of the female.

Consistently with the predictions for otariids discussed above, the δ^{13}C and δ^{15}N values of the captive female South American sea lion here studied increased slightly from parturition to weaning in the two lactation cycles monitored. But pregnancy had an even much larger impact on the δ^{13}C values of the female than lactation, which resulted in the absence of correlation between δ^{13}C and δ^{15}N values when the whole vibrissae is considered. In this situation, discriminating between physiological and dietary sources of variability in wild otariids can be difficult. The ten adult wild South American sea lions here studied exhibited periodical oscillations of δ^{13}C and δ^{15}N along their vibrissa, as well as the females studied by Baylis et al. (2015) in the Falkland/Malvinas Islands. In males, fasting during the rutting period likely explains most of the variability in the δ^{15}N values, but the variability in the δ^{13}C values was larger than expected and probably related to dietary shifts. The impact of dietary shifts was probably even larger in females, not only because the variability of δ^{13}C and δ^{15}N was larger than expected from the breeding cycle alone, but also because the δ^{13}C and δ^{15}N values oscillated simultaneously, something which was not true in captivity.

Drago et al (2010) proposed that southern sea lion females from northern Patagonia shift from pelagic/offshore prey to demersal/onshore prey after parturition. In northern Patagonia, pelagic prey are depleted in ^{13}C and ^{15}N as compared to benthic prey (Drago et al. 2010) and hence the δ^{13}C and δ^{15}N values in the female vibrissa were expected to fluctuate simultaneously if such a cyclical dietary shift was true and prevailed over the physiological changes related to the annual breeding cycle.

The existence of a simultaneous oscillation in wild females, but not in captive females, is consistent with this hypothesis. Furthermore, parallel oscillations of similar amplitude have been reported in females exploiting coastal foraging grounds off the Falkland/Malvinas Islands, whereas the oscillations observed in females exploiting offshore grounds had lower amplitude (Baylis et al. 2015). This is also consistent with the existence of pelagic/offshore-demersal/onshore migration during the annual cycle in the coastal foragers. Nevertheless, the values of both stable isotope ratios reported in the present study were usually within the range expected from local prey, after accounting for the trophic discrimination factor derived from the captive animals, and hence migration to distant foraging grounds can be ruled out. Only in the youngest female (OF 1068) the increasing trend of the $\delta^{13}C$ values observed along the length of vibrissa might be indicative of increased consumption of benthic prey as she grew older, as previously reported (Drago et al. 2009).

In conclusion, both dietary shifts and the physiological processes related to the breeding cycle may influence the stable isotope values in the vibrissae of otariids and probably other metabolically inert tissues. Thus, caution is needed when interpreting variability solely as evidence of dietary or habitat shifts.

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TERCER CAPÍTULO

EFECTOS DE LA EXPLOTACIÓN HUMANA SOBRE LA DIETA DE LOS LOBOS MARINOS SUDAMERICANOS

Cambios en el nicho trófico de los depredadores marinos: la dieta del lobo marino sudamericano (*Otaria flavescens*) desde finales del Holoceno hasta el presente, como un caso de estudio

RESUMEN. En el presente estudio, se analizaron las razones isotópicas de carbono y nitrógeno de muestras arqueológicas y modernas de hueso de lobo marino común (*Otaria flavescens*) para reconstruir los cambios en su dieta en el Atlántico sudoccidental del desde el Holoceno tardío hasta la actualidad en el Atlántico sudoccidental. Las muestras óseas procedían de concheros situados en el centro-norte y sur de Patagonia, en Argentina, y de colecciones científicas modernas. También se analizaron las razones isotópicas de conchas de moluscos procedentes de yacimientos arqueológicos y modernos procedentes del intermareal rocoso a fin de explorar posibles cambios en la línea de base del paisaje isotópico. De este modo se pueden comparar los valores isotópicos de muestras de hueso de diferentes períodos, una vez corregidos en función de la variación de la línea de base. Los resultados confirmaron la plasticidad trófica del lobo marino sudamericano, revelando que el impacto sobre su dieta provocado por los cambios demográficos derivados de su explotación moderna fueron muy superiores a los originados durante la explotación por parte de los cazadores-recolectores Estas conclusiones se ven corroboradas por la estabilidad de la dieta de los lobos marinos sudamericanos durante varios milenios de explotación aborigen, tanto en la Patagonia norte-centro con en la Patagonia sur, en contraste con el drástico aumento del nivel trófico de la especie durante el XX. Además se destaca que este aumento en el nivel trófico podría estar relacionado con una disminución del tamaño poblacional, como resultado de la explotación comercial, y una disminución de la competencia intraespecifica. Finalmente estos resultados confirman lo mucho que se puede aprender sobre la ecología de las especies gracias a estudios retrospectivos, pues en la situación actual la estructura de ecosistemas ha sido modificada por los impactos humanos y es totalmente diferente a la de los ecosistemas prístinos donde evolucionaron las especies actuales.

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Shifting niches of marine predators due to human exploitation: the diet of the South American sea lion (*Otaria flavescens*) since the late Holocene as a case study

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Abstract.——Stable isotope ratios of carbon and nitrogen in archaeological and modern bone samples have been used to reconstruct the dietary changes of the South American sea lion *Otaria flavescens* from the late Holocene to the present in the southwestern Atlantic. We sampled bones from archaeological sites in northern-central and southern Patagonia, Argentina, and bones housed in modern scientific collections. Additionally, we analyzed the stable isotope ratios in ancient and modern shells of intertidal molluscs to explore changes in the isotope baseline and allow comparison between bone samples from different periods after correction for baseline shifts. Results confirmed the trophic plasticity of the South American sea lion, demonstrated the much larger impact of modern exploitation of marine resources as compared with that of hunter-gatherers, and underscored the dissimilarity between the past and modern niches of exploited species. These conclusions are supported by the rather stable diet of South American sea lions during several millennia of aboriginal exploitation, in both northern-central and southern Patagonia, and the dramatic increase in trophic level observed during the twentieth century. The recent increase in trophic level might be related to the smaller population size resulting from modern sealing and the resulting reduced intraspecific competition. These results demonstrate how much can be learned about the ecology of modern species thanks to retrospective studies beyond the current, anthropogenically modified setting where ecosystem structure is totally different from that in the pristine environments where current species evolved.

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Introduction

Humans have dramatically modified the world by altering biogeochemical cycles and eroding biodiversity (Crutzen 2002; Terborgh and Estes 2010). Large species are particularly vulnerable to human impacts, and many of them declined in population size, or went extinct, before we had the ability to study and understand their ecological significance (Jackson et al. 2001; Martin 2005; Terborgh and Estes 2010). Nevertheless, biologists still attempt to interpret the biology of extant species as if they had evolved in the current anthropogenic setting (Steadman 2006). In this context, a historical reference point is essential not only for understanding the evolutionary processes that operated in pristine environments, but also for providing goals for restoration and management (Jackson et al. 2001; Terborgh and Estes 2010; Braje and Rick 2011). Although much of the anthropogenic loss of biodiversity has been caused by the expansion of western societies, aboriginal exploitation also resulted in significant levels of extinction and habitat modification (Jackson et al. 2001; Martin 2005; Terborgh and Estes 2010). Thus, we must study deep time, using the stable isotope ratios in bone collagen to reconstruct paleodiets and trophic levels to understand the natural trophic ecology of modern species in their original environments (Ambrose and DeNiro 1989; Schwarcz 2000).

Human impacts are not limited to terrestrial ecosystems; the world oceans also have been severely affected (Halpern et al. 2008). Eared seals, or Otariids, may exert strong top-down effects on ecosystem structure where abundant enough (Yodzis 1998; Koen-Alonso and Yodzis 2005) and are still major components of coastal ecosystems in the temperate regions of the Southern Hemisphere (Gentry 2009). However, most species of eared seals, commercially exploited for their pelts and fat, were hunted to the brink of extinction throughout the nineteenth and twentieth centuries, and currently some species remain well below their original numbers (Kovacs et al. 2012). Nevertheless, in some regions the human exploitation of eared seals predates the arrival of western sealers by several millennia, thus raising many questions about the actual impact of aboriginal exploitation (Porcasi et al. 2000; Jones et al. 2004; Newsome et al. 2007; Tivoli and Zangrando 2011).

The South American sea lion *Otaria flavescens* is widely distributed along some 10,000 km of the coast of South America (Cappozo and Perrin 2009). According to the zooarchaeological record, pinnipeds and other marine species were widely exploited by the hunter-gatherers inhabiting the Beagle Channel and northern Patagonia during the late Holocene, although the effect of aboriginal hunting on their populations remains uncertain (Schiavini 1993; Orquera and Piana 1999; Gómez Otero 2006; Favier Dubois et al. 2009; Favier Dubois and Kokot 2011; Orquera et al. 2011; Tivoli and Zangrando 2011; Borella and Cruz 2012; Favier Dubois and Scartascini 2012). Modern exploitation through its entire range began in the eighteenth century and lasted until the first half of the twentieth century, by which time the species had been severely depleted (Cappozo and Perrin 2009). In Uruguay, the population is still decreasing, even though sealing ceased in 1995 (Páez 2006). The population breeding in Argentina experienced a 90% decline from the 1920s to the 1960s and currently is approximately one-third of the original size (Crespo and Pedraza 1991; Dans et al. 2004; Schiavini et al. 2004). Simultaneously, numbers of sea lions declined in the Falkland (Malvinas) Islands, where the small remaining population is less than 1.5% of the original size (Thompson et al. 2005).

South American sea lions are no longer hunted in the region, but the intense exploitation of fishes, squids, and crustaceans experienced during the last 40 years has deeply modified the structure of the ecosystems and caused a dramatic decrease in average fish size (Koen-Alonso and Yodzis 2005; Dato et al. 2006). South American sea lions are generalist predators (Thompson et al. 1998; Koen Alonso et al. 2000; Campagna et al. 2001; Riet-Sapriza et al. 2012) and during the twentieth century they have shifted their diet to adapt to the new environmental conditions created by industrial fishing (Suárez et al. 2005; Drago et al. 2009; Romero et al. 2011), but at the cost of consuming less profitable prey and reducing body size (Drago et al. 2010). Nevertheless, intraspecific competition, and not competition with fisheries, has been proposed to be the major determinant of diet composition in South American sea lions (Drago et al. 2009, 2010), as they consume primarily large prey that feed near the bottom (demersal hereafter) when and where the population is small (Koen Alonso et al. 2000; Drago et al. 2009; Riet-Sapriza et al. 2012) and shift to smaller benthic and pelagic prey when and where the population increases (Suárez et al. 2005; Drago et al. 2009; Romero et al. 2011). If this hypothesis is true, the current trophic niche of South American sea lions would be different from that exploited in the past, when the population was much larger (Rodriguez and Bastida 1998; Dans et al. 2004; Schiavini et al. 2004).

The presence of bones of the South American sea lion in the zooarchaeological record, together with extensive scientific collections of modern skeletal material, offers a unique opportunity to assess the magnitude of change in the trophic ecology of a marine top predator in response to human exploitation and compare the effect of aboriginal and modern exploitation. To do so, here we analyze the stable isotope ratios of carbon and nitrogen in the bone of ancient and modern South American sea lions from Argentina to assess the dietary changes of the species through the second half of the Holocene and determine the trophic niche of the species in ancient ecosystems. The stable isotope ratios of modern and ancient organisms cannot be compared directly, because temporal variations in the isotopic baseline may exist (Casey and Post 2011). Nonetheless, the proteins that make up the organic matrix of mollusc shells can become encased within mineral crystals and preserved, hence offering a material suitable to reconstructing the changes in the isotopic baseline (Crenshaw 1980; Bailey et al. 2008; Casey and Post 2011). Accordingly, we also analyzed the stable isotope ratios in the shells of modern and ancient limpets and mussels to reconstruct, and compensate for, changes through time in the stable isotope baseline.

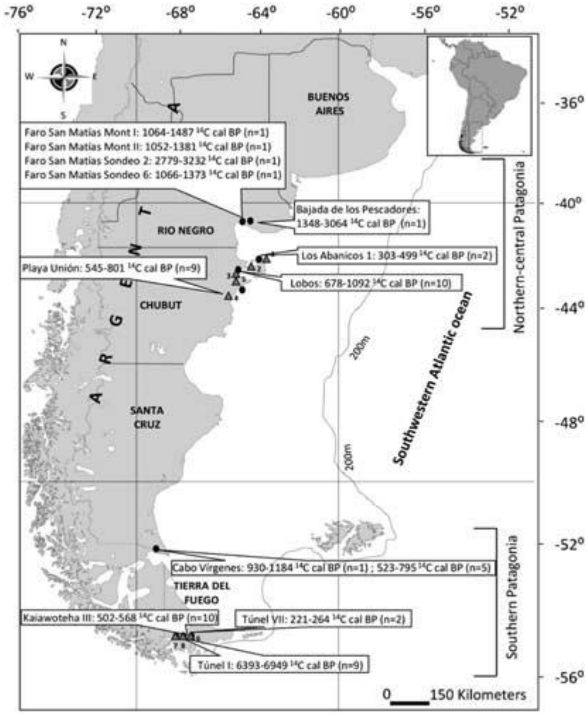

FIGURE 1. Location of archaeological sites from which sea lions and shells were sampled for stable isotope analysis. Sample sizes are listed in parentheses. The filled circles show archaeological sites for sea lions and the triangles denote sites for shells: 1 = Los Abanicos 1; 2 = Las Ollas Conchero 1; 3 = Ecocentro Fogón 3; 4 = Playa Las Lisas 2; 5 = Cracker 6; Túnel VII; 7 = Shamakush X; 8 = Imiwaia I.

Material and Methods

Study Site and Sample Collection.—We measured the stable isotope ratios of carbon and nitrogen of both modern and archaeological bone collagen samples of South American sea lions from two areas in Argentina (Fig. 1), northern-central Patagonia (from 39°S to 46°S) and southern Patagonia (from 46°S to 55°S). Modern samples of turbinate bones from South American sea lions were collected from specimens at the scientific collections at Centro Nacional Patagónico (Puerto Madryn, Argentina) and Museo Acatushún (Ushuaia, Argentina); the corresponding stable isotope ratios of carbon and nitrogen had been published previously elsewhere (Drago et al. 2009). Zooarchaeological bone samples from different skeletal elements were recovered from different layers of shell middens in northern-central Patagonia and southern Patagonia by researchers from Centro Nacional Patagónico, Centro Austral de Investigaciones Científicas, and Instituto Multidisciplinario de Historia y Ciencias Humanas (Table 1).

The samples were dated in different laboratories and using different methods, in particular samples from northern-central Patagonia, where all dated samples were marine shells instead of charcoal. We calibrated radiocarbon ages using the package Clam 2.2 (Blaauw 2010) and the new curve for Southern Hemisphere ShCal13 (Hogg et al. 2013). Reservoir effects data for the northern Patagonia region have emerged only recently, and they suggest variable differences between marine and terrestrial ages (Cordero et al. 2003; Favier Dubois 2009).

From December 2009 to February 2010 we collected the shells of modern molluscs from the two study regions (Supplementary Table). We have also analyzed zooarchaeological shell samples recovered from different layers of shell middens in northern-central Patagonia and the Beagle Channel, Tierra del Fuego (Fig. 1). Clementz and Koch (2001) pointed out that five samples are enough to provide robust estimates of mean and standard deviation for stable

TABLE 1. Ratios of stable isotopes of carbon and nitrogen in the bone tissue of South American sea lions from the archaeological sites of northern-central Patagonia (Río Negro and Chubut) and southern Patagonia (Santa Cruz and Tierra del Fuego).

Sample ID	Archaeological site	Skeletal element	Age (^{14}C ybp)	Calibrated age (^{14}C cal BP (±1σ)^1	δ^{13}C (‰)	δ^{15}N (‰)	C:N	Reference
northern-central Patagonia								
FSM-SRH	Faro San Matías Mont II	rib	1630 ± 80	1052–1381 (91.1%)^1	−11.4	22.4	3.3	Borella and Cruz 2012; Favier Dubois et al. 2009
OBS 13	Faro San Matías 6	vertebra	1380 ± 80	1066–1373 (95%)	−11.2	22.4	3.1	
OBS 137	Bajada de los pescadores	sternum	2197 ± 38	1348–3064 (94.9%)	−13.4	21.1	3.6	
OBS 4	Faro San Matías 2	radius	2910 ± 90	2779–3232 (95%)	−13.9	18.1	3.6	
FSM-SRH	Faro San Matías Mont I	rib	1680 ± 90	1064–1487 (94.2%)^1	−11.9	23.0	3.7	
36	Los Abanicos 1	elbow	380 ± 60	303–499 (95%)	−12.3	21.4	4.0	Gómez Otero 2006
82	Los Abanicos 1	elbow			−11.8	23.8	3.5	
F_1 17	Playa Union	humerus	1040 ± 70	545–801 (94.6%)^1	−13.7	19.9	3.3	Gómez Otero 2006
$FM_1$13	Playa Union	humerus			−13.1	19.7	4.1	
$FM_1$14	Playa Union	humerus			−13.4	21.0	3.3	
$FM_1$15	Playa Union	humerus			−12.1	20.3	3.0	
$FM_1$16	Playa Union	humerus			−12.3	18.7	3.3	
$FM_1$17	Playa Union	humerus			−12.7	18.9	3.2	
$FM_1$19	Playa Union	humerus			−12.9	22.5	3.3	
$FM_1$20	Playa Union	humerus			−12.3	21.9	3.2	
$FM_1$21	Playa Union	humerus			− 12.8	20.3	3.4	
i_1 61	Lobos*	humerus	1290 ± 100	678–1092 (94.2%)^1	−13.1	21.3	3.8	Serrán et al. 2008
i_3 43	Lobos*	humerus			−13.0	22.0	3.2	
i_4 44	Lobos*	humerus			−13.4	22.3	3.7	
i_5 54	Lobos*	humerus			−11.0	22.2	3.4	
I_{15} C1	Lobos*	humerus			−14.4	22.2	2.7	
I_{18} 89	Lobos*	humerus			−13.6	25.0	3.4	
i 23	Lobos*	humerus			−12.8	21.3	3.3	
i 24	Lobos*	jaw			−14.6	23.2	3.5	
$M_1$1	Lobos*	humerus			−12.0	22.3	3.0	
$M_1$3	Lobos*	humerus			−13.0	22.4	3.8	
southern Patagonia								
CV6 4/-60–65cm	Cabo Vírgenes	rib	1190 + 60	930–1184 (94.7%)^2	−13.9	20.8	3.4	L' Heureux and Franco 2002
CV20 OF1	Cabo Vírgenes	phalange			−14.8	15.4	3.0	
CV20 OF3	Cabo Vírgenes	skull			−14.5	17.9	3.4	
CV20 OF4	Cabo Vírgenes	skull	1256 ± 50	523–795 (95%)^2	−12.2	19.1	3.2	Belardi et al. 2011
CV 20 OF6	Cabo Vírgenes	jaw			−12.3	19.6	3.9	
CV20 OF7	Cabo Vírgenes	skull			−14.2	18.8	3.5	
30459	Túnel I, Capa D	jaw	5000–4300	6393–6949 (93.2%)	−11.3	19.6	3.2	Orquera and Piana 1988
33458	Túnel I, Capa D	jaw			−12.4	18.5	3.0	
33459	Túnel I, Capa D	jaw			−11.4	18.7	3.2	
33551	Túnel I, Capa D	jaw			−11.8	18.8	3.2	
33571	Túnel I, Capa D	jaw			−12.5	19.0	3.4	
33717	Túnel I, Capa D	jaw			−12.7	18.4	2.7	

34177	Túnel I, Capa D	jaw			−12.3	18.6	3.1	
34544	Túnel I, Capa D	jaw			−12.0	18.4	3.9	
34751	Túnel I, Capa D	jaw			−11.6	18.9	3.8	
43418	Túnel VII	bone**	100 ± 45	221–264 (17.0%)	−11.7	18.5	2.7	Piana et al. 1992
154286	Túnel VII	bone**			−11.3	17.7	2.8	
OF 2a	Kaiawoteha III, Capa	humerus	580 ± 45	502–568 (74.2%)	−13.8	17.4	3.5	Piana et al. 2007
OF 2b	K Kaiawoteha III, Capa	humerus			−15.6	16.8	3.2	
OF 3	K Kaiawoteha III, Capa	coxal			−15.1	16.5	2.9	
OF 5	K Kaiawoteha III, Capa	radius			−15.6	16.9	3.4	
OF 7	K Kaiawoteha III, Capa	radius			−14.2	17.6	3.7	
OF 9	K Kaiawoteha III, Capa	humerus			−14.2	16.3	3.9	
OF 10	K Kaiawoteha III, Capa	coxal			−12.6	17.3	4.0	
OF 12	K Kaiawoteha III, Capa	maxilla			−12.2	17.8	4.1	
OF 14	K Kaiawoteha III, Capa	coxal			−13.2	17.8	3.3	
OF 15	K Kaiawoteha III, Capa	maxilla			−14.1	19.2	3.2	

*Paleontological site

**Unspecified

[1] The regional marine reservoir effect of 266 ± 51 years was included in the calibration of the samples (Favier Dubois 2009).

[2] The regional marine reservoir effect of 516 ± 85 years was included in the calibration of the samples (Cordero et al. 2003).

isotope ratios in tissues that integrate dietary information over long periods of time, and hence sample size was set at five for each species, locality, and zooarchaeological stratum where available (Supplementary Table). The limpet *Nacella magellanica* was sampled everywhere, but the rubbed mussel (*Aulacomya atra atra*) was sampled in northern-central Patagonia and the blue mussel (*Mytilus edulis*) in southern Patagonia, according to availability in regional shell middens.

Bones of some fish species are abundant in the zooarchaeological record of both northern-central and southern Patagonia (Favier Dubois et al. 2009: Favier Dubois and Kokot 2011; Favier Dubois and Scartascini 2011; Tivoli and Zangrando 2011), but the remains of cephalopods, shrimp and squat lobsters are missing. These taxa are important prey for modern South American sea lions (Thompson et al. 1998; Koen Alonso et al. 2000; Suárez et al. 2005; Romero et al. 2011) and hence necessary for comparisons between the stable isotope ratios of ancient South American sea lions and those of potential prey from the same period and region. For this reason, we analyzed muscle samples from the prey species currently consumed by South American sea lions (Table 2) and inferred the likely stable isotope ratios expected for ancient prey after correcting for the changes in the isotopic baseline revealed by the analysis of mollusc shells. Furthermore, we computed a diet-to-bone discrimination factor by combining published information about diet-to-vibrissa fractionation in marine carnivores (Hobson et al. 1996; Newsome et al. 2010) and the stable isotope ratios of paired samples of vibrissa and bone from eight adult South American sea lions dead-stranded in northern Patagonia between 2006 and 2011 (see below for details about the calculations). This discrimination factor is necessary for comparing the stable isotope rations in the tissue of the predator with those in the tissue of its prey.

Bone and shell samples were stored dry at room temperature. Samples from potential prey were stored at −20°C prior to analysis.

Stable Isotope Analysis.—Bones were cleaned of sediment and dried in a stove at 50°C. Shell samples were polished with sandpaper

TABLE 2. Ratios of stable isotopes of carbon and nitrogen (mean ± standard deviation) in the muscle of modern potential prey of the South American sea lion off northern-central Patagonia and southern Patagonia.

Sample					
Species	Common name or synomym	Family	*n*	δ13C (‰) (mean ± SD)	δ15N (‰) (mean ± SD)
northern-central Patagonia					
Demersal					
Enteroctopus megalocyathus	Red octopus	Octopodidae	4	−14.7 ± 0.6	18.5 ± 1.9
Genypterus blacodes	Pink cusk-eel	Ophidiidae	5	−14.7 ± 0.4	18.0 ± 0.3
Munida subrugosa	Squat lobster	Galatheidae	5	−15.7 ± 0.8	16.8 ± 0.4
Octopus tehuelchus	Tehuelchus	Octopodidae	5	−14.8 ± 0.2	19.9 ± 0.4
Paralichthys isosceles	Flounder	Paralichthyidae	5	−16.0 ± 0.4	18.0 ± 0.6
Patagonotothen cornucola	*Notothenia cornucola*	Nototheniidae	2	−15.6 ± 0.1	18.8 ± 0.2
Patagonotothen ramsayi	*Notothenia ramsayi*	Nototheniidae	2	−16.1 ± 0.2	18.6 ± 0.2
Platyxanthus patagonicus	True crabs	Platyxanthidae	5	−15.3 ± 0.2	16.3 ± 0.9
Pleoticus muelleri	Red shrimp	Solenoceridae	5	−15.9 ± 0.4	16.7 ± 0.3
Raneya brasiliensis	Banded cusk-eel	Ophidiidae	5	−15.3 ± 0.7	18.8 ± 0.5
Pelagic					
Engraulis anchoita	Argentine anchovy	Engraulidae	5	−18.0 ± 0.2	15.7 ± 0.8
Illex argentinus	Argentine short-finned squid	Ommastrephidae	5	−17.0 ± 0.6	13.7 ± 0.8
Loligo gahi	Patagonian squid	Loliginidae	5	−17.2 ± 1.0	16.2 ± 1.2
Loligo sanpaulensis	Brazilian squid	Loliginidae	5	−16.8 ± 0.2	17.2 ± 0.3
Merluccius hubbsi (≥30cm)	Argentine hake	Merlucciidae	9	−17.3 ± 0.7	16.2 ± 0.5
Merluccius hubbsi (<30cm)	Argentine hake	Merlucciidae	5	−17.0 ± 0.5	17.1 ± 0.4
Stromateus brasiliensis	Southwest Atlantic butterfish	Stromatidae	2	−17.0 ± 0.9	16.2 ± 0.4
southern Patagonia					
Demersal					
Eleginops maclovinus	Patagonian blenny	Eleginopsidae	2	−16.5 ± 0.1	18.4 ± 0.4
Genypterus blacodes	Pink cusk-eel	Ophidiidae	5	−17.2 ± 0.8	18.3 ± 0.5
*Macroronus magellanicus**	Hoki	Merlucciidae	5	−18.2 ± 0.7	13.9 ± 0.7
Munida gregaria	Lobster krill	Galatheidae	5	−15.4 ± 2.6	11.9 ± 1.1
Paralichthys patagonicus	Patagonian flounder	Paralichthyidae	1	−15.8	19.0
Patagonotothen ramsayi	Rock cod	Nototheniidae	5	−18.4 ± 1.4	15.6 ± 2.2
Pleoticus muelleri	Red shrimp	Solenoceridae	5	−15.6 ± 0.4	15.7 ± 0.5
Psammobatis rudis	Smallthorn sand skate	Rajidae	3	−14.8 ± 0.8	16.7 ± 0.6
Pelagic					
Illex argentinus	Argentine short-finned squid	Ommastrephidae	5	−17.9 ± 0.4	16.4 ± 0.7
Loligo gahi	Patagonian squid	Loliginidae	5	−17.2 ± 1.0	17.3 ± 2.1
Merluccius hubbsi (≥30 cm)	Argentine hake	Merlucciidae	10	−18.0 ± 0.2	17.2 ± 0.2
Merluccius hubbsi (<30 cm)	Argentine hake	Merlucciidae	3	−16.8 ± 0.2	16.7 ± 0.3
*Mictophids	Lanternfish	Myctophidae	17	−21.9 ± 0.7	11.7 ± 0.9
Sprattus fuegensis	Falkland sprat	Clupeidae	5	−17.8 ± 0.3	17.2 ± 0.2
Stromateus brasiliensis	Southwest Atlantic butterfish	Stromatidae	5	−17.8 ± 0.5	15.7 ± 1.2

*Reference: Ciancio et al. 2008

and with a diamond wheel drill to remove impurities and subsequently rinsed with distilled water and dried in a stove at 50°C. White muscle from fish and mantle from squids were thawed and dried in a stove at 50°C. Once dry, all samples were ground to a fine powder with a mortar and pestle. Because shells and bone contain high concentrations of inorganic carbon, which may bias δ13C values (Lorrain 2003), they were divided in two aliquots. One of them was decarbonized by soaking during in 0.5 N (bone) or 1 N (shell) hydrochloric acid (HCl) until no more CO_2 was released (Newsome et al. 2006). The HCl treatment adversely affects δ15N values (Bunn et al. 1995), so the other aliquot was not treated with HCl and was used for δ15N determination. Lipids were extracted from bone samples with a chloroform / methanol (2:1) solution (Bligh and Dyer 1959).

The vibrissae were washed in methanol in an ultrasonic bath for 20 min in order to remove residual deposits or any lipid contamination from the vibrissae's surface as a result of handling, and then were dried again for 48 hr at 50°C. Vibrissae were cut into 3-mm-long consecutive sections starting from the proximal end. This is because each section integrates diet during one month (Hirons et al. 2001)

Approximately 0.8 mg of bone, 0.3 mg of vibrissae, 0.4–9.9 mg of shell, and 0.3 mg of white muscle from fish and mantle from cephalopods were weighed into tin cups (3.3 × 5 mm), combusted at 900°C, 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 Centres Cientifics i Tecnològics de la Universitat de Barcelona. The samples from modern South American sea lions had already been analyzed in the same laboratory and the results had been reported by Drago et al. (2009).

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

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N, and R_{sample} and R_{standard} are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the sample and standard, respectively. The standards used were Vienna Pee Dee Belemnite (VPDB) calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N.

Stable Isotope Discrimination Factors.— Animals are related isotopically to their environment by means of an isotopic diet-tissue discrimination factor (Hobson 1999). These factors vary significantly, within and between species, with diet, physiology, and tissue (Gannes et al. 1997; Olive et al. 2003; Koch 2007). Discrimination factors from diet to enamel and bone have been assessed in ungulates (Passey and Cerling, 2002; Nardoto et al. 2006), but they are unlikely to be useful because enamel and bone may differ in fractionation factors (Riofrío-Lazo and Aurioles-Gamboa 2013) and nutrient routing is different between omnivores and carnivores (Martínez del Rio et al. 2009). For this reason, we have computed a diet-to-bone fractionation factor using published information about diet-to-vibrissa fractionation in marine carnivores (Hobson et al. 1996; Newsome et al. 2010) and comparing the stable isotope ratios of vibrissa and bone of South American sea lions (eq. 2), as bone is expected to integrate diet over several years (Newsome et al. 2006) and the same is true for long otariid vibrissa, with each few millimeters corresponding to several weeks (Cherel et al. 2009):

$$\Delta_{(\text{bone-vibrissae})} + \Delta_{\text{Means Reference}}(\text{vibrissae-diet}) = \Delta(\text{bone- diet}) \qquad (2)$$

Data Analysis.—The $\delta^{13}C_{\text{shell}}$ and $\delta^{15}N_{\text{shell}}$ values of limpets and mussels allowed tracking changes in the stable isotope baseline through time. The $\delta^{13}C_{\text{shell}}$ and $\delta^{15}N_{\text{shell}}$ values of modern and ancient individuals of each species from the same region were compared using the nonparametric Kruskal-Wallis test for multiple comparisons, because the assumptions of normality (using Lilleford test) and homoscedasticity (using Leven test) were seldom met (Zar 1984).

Stable isotope ratios in archaeological and modern bone samples were compared only after correcting for changes in the isotopic baseline (Casey and Post 2011). When statistically significant differences were found between modern and ancient stable isotope ratios, a correction factor was computed as the difference between the average stable isotope ratio of modern and ancient shells from each locality and age. Secondly, the difference was added to the stable isotope ratio of ancient bones from the same locality and age, to allow comparison with modern samples. For instance, if the $\delta^{15}N$ value of modern shells was 2‰ above that of ancient ones, the $\delta^{15}N$ value of ancient bones had to be increased 2‰ to be compared with that of modern bones. When bones came from a stratum without associated mollusc shells, bone stable isotope ratios were corrected using the time-weighted average of the correction factors computed for nearest strata below and above. Ideally, a bottom grazer (limpet) and a suspension feeder (ribbed mussel and blue mussel) were combined from each locality, but this was not always possible. A detailed description of those calculations and the resulting correction factors are shown in Table 3.

Once we had corrected for isotope baseline shifts, we compared stable isotope ratios in

TABLE 3. Baseline correction factor for shells and sea lions to each radiocarbon year (ybp) where we obtained samples. Underlined numbers are the correction factors utilized for calculating the weighted values.

Age $_{shell}$ (^{14}C ybp)	Correction Factor (shell) (Δ = δ^{13}C or $\delta^{15}N_{14C\ age\ BP\ shell}$ - δ^{13}C or $\delta^{15}N_{0BP}$) Δ δ^{13}C		Δ δ^{15}N		Average correction factors (shell) Δ δ^{13}C	Δ δ^{15}N	Age $_{bone}$ (^{14}C ybp)	Correction factors used for baseline Δ δ^{13}C	Δ δ^{15}N
northern-central Patagonia									
	Aulacomya atra atra	*Nacella magellanica*	*Aulacomya atra atra*	*Nacella magellanica*					
380 ± 60	1.5	−0.9	2.4	3.6	0.3	3.0	380 ± 60	0.3	3.0
610 ± 60	−0.1	–	1.6	–	−0.1	1.6	1380 ± 80	-0.4^{*1}	1.8^{*2}
640 ± 60							1000 ± 70		
850 ± 50	–	−1.6	–	1.7	−1.6	1.7	1630 ± 80		
2140 ± 50	1.8	–	2.2	–	1.8	2.2	1680 ± 90		
2600 ± 60	–	−1.7	–	3.8	−1.7	3.8	2197 ± 38	0.1^{*3}	3.0^{*4}
5200 ± 70	−1.8	−4.1	2.6	2.3	−2.9	2.5	2910 ± 90	−1.7	3.8
^{*1,2} weighted value calculating by: ^{1} (0.63 * − 1.6 + 0.33 * 1.8); ^{2} (0.63 * 1.7 + 0.33 * 2.2)									
^{*3,4} mean values calculating by: ^{3} 1.8 and − 1.7; ^{4} 2.2 and 3.8									
southern Patagonia									
100 ± 45	*Mytilus edulis* −3.7	*Nacella magellanica* −6.7	*Mytilus edulis* 1.5	*Nacellla magellanica* 1.7	−5.2	1.6	100 ± 45	−5.2	1.6
500 ± 100	2.1	−0.3	1.6	1.3	0.9	1.8	580 ± 45	0.9	1.5
890	3.4	–	–	–	−3.4	1.5	1256 ± 50–1190 ± 60	$-3.4^{\#1}$	$1.7^{\#2}$
5940–5710	−1.5	−5.8	3.5	2.6	−3.6	3.1	5000–4300	−3.6	3.1

^{1,2} weighted value, calculated by: ^{1}(0.90 *−3.4 + 0.10 * −3.6); ^{2} (0.9 *1.5 + 0.10 *3.1) bone samples with those of modern potential prey, after applying the diet-to-bone discrimination factors for South American sea lions ($\Delta\delta^{13}C = 3.5 \pm 0.8‰$; $\Delta\delta^{15}N = 4.4 \pm 0.8‰$) obtained in this study. Mann-Whitney *U*-tests were used for testing differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures between demersal and pelagic modern prey.

Data are presented as mean ± standard deviation (SD) and significance was assumed at the 0.05 level. All statistical analyses were carried out with PASW Statistics (Version 17.0 for Windows, SPSS).

Results

Although the $\delta^{13}C_{shell}$ values of the mussels and limpets from northern-central Patagonia did not vary throughout the late Holocene (Fig. 2; Kruskal-Wallis test; *A. atra atra*: $\chi^2 = 9.418$, df = 4, $p = 0.052$; *N. magellanica*: $\chi^2 = 5.352$, df = 4, $p = 0.253$), those of the mollusc species collected in southern Patagonia exhibited a remarkable variability and changes run in parallel in both species (Fig. 2; Kruskal-Wallis test; *M. edulis*: $\chi^2 = 15.714$, df = 4, $p = 0.003$; *N. magellanica*: $\chi^2 = 11.765$, df = 4, $p = 0.008$). Likewise, differences through time in the $\delta^{15}N_{shell}$ values of mussels and limpets were statistically significant both in northern-central Patagonia (Kruskal-Wallis test; *A. atra atra*: $\chi^2 = 19.549$, df = 4, $p < 0.001$; *N. magellanica*: $\chi^2 = 17.000$, df = 4, $p = 0.002$) and in southern Patagonia (Kruskal-Wallis test; *M. edulis*: $\chi^2 = 15.684$, df = 4, $p = 0.001$; *N. magellanica*: $\chi^2 = 14.392$, df = 4, $p = 0.002$), and the two species from the same area exhibited the same pattern of temporal variation (Fig. 2) although each region evolved independently. These results revealed major changes in the stable isotope baseline, and hence correction factors were computed for each region and period to allow comparison of the stable isotope ratios in the bone of ancient and modern South American sea lions (Table 3).

Vibrissae were depleted both in ^{13}C and ^{15}N relative to bone (mean $\delta^{13}C$: vibrissae = − 13.1 ± 0.8; bone = − 12.3 ± 0.8; mean $\delta^{15}N$: vibrissae = 21.2 ± 0.9; bone = 22.5 ± 1.5; $p = 0.01$), which resulted in an average vibrissa-to-bone discrimination factor of 0.8 ± 0.8‰ for $\delta^{13}C$ and

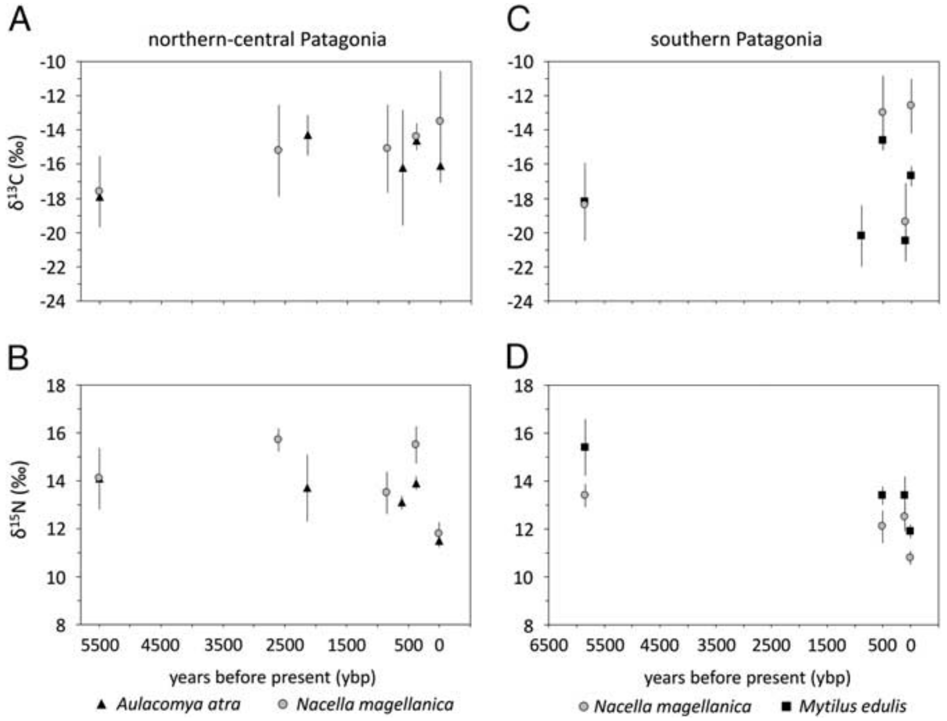

FIGURE 2. Temporal trends throughout the late Holocene of the $\delta^{13}C_{shell}$ and $\delta^{15}N_{shell}$ values is molluscs from northern-central Patagonia and southern Patagonia. Differences through time were statistically significant; except for the $\delta^{13}C_{shell}$ values of *Aulacomya atra atra* and *Nacella magellanica* from northern Patagonia (see the text for details about the statistical analyses).

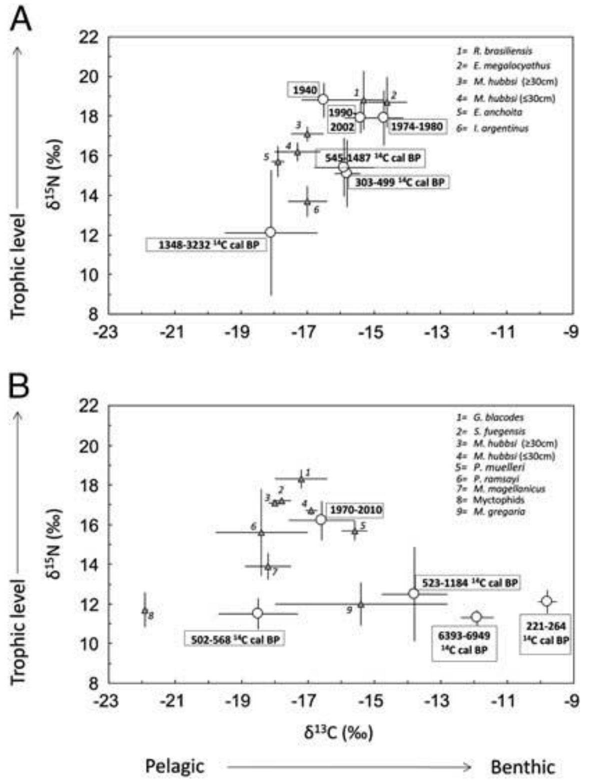

FIGURE 4. Bi-plot of the isotopic signal of the South American sea lion from northern-central (A) and southern (B) Patagonia, after correcting for differences in isotope baseline and for the diet-to-bone fractionation. Circles denote sea lions from different periods and triangles denote main prey.

squat lobster *Munida gregaria* were more depleted in ^{15}N than any other species. Accordingly, the low $\delta^{15}N_{bone}$ values typical of ancient South American sea lions suggest a diet dominated by prey at a low trophic level like *M. gregaria* (Fig. 4), whereas modern South American sea lions consume a larger proportion of prey at a higher trophic level like the rock cod *Patagonotothen ramsayi,* the Argentine hake *Merluccius hubbsi*, and the Argentine shrimp *Pleoticus muelleri*. Interestingly, the two samples from the nineteenth century fell outside the mixing polygon, suggesting they had a totally different diet or came from an area with a different isotopic baseline.

Discussion

The overall evidence reported here indicates that South American sea lions currently forage ~~at a higher trophic level than they did during~~ the late Holocene. Furthermore, those inhabiting northern-central Patagonia forage more benthically than they used to do in the past. Ignorance about the actual age and sex of the ancient South American sea lions recovered from the shell middens, the analysis of different skeletal elements, and the inference made about the stable isotope ratios of ancient prey species might bias the results (Balasse et al. 1999), but the difference between ancient and modern stable isotope ratios is so large that current South American sea lions certainly occupy a totally different trophic level, both in northern-central and southern Patagonia.

The data also reveal the twentieth century as the period when most of the change in the trophic level of South American sea lions happened, following the massive removal of individuals by hunting along the coast of Argentina (Dans et al. 2004; Schiavini et al. 2004; Grandi et al. 2012). Major changes also occurred in southern Patagonia during the early nineteenth century, as all the samples fell outside the mixing polygon formed by the stable isotope ratios of modern prey, even after correction for a shift in the isotope baseline. Western sealing had actually decimated otariid populations in the southern Patagonia during the late eighteenth and the early nineteenth centuries and only isolated otariids were sporadically recorded in the region during the second half of the nineteenth century (Bridges 1949). Rookeries persisted on Staten Island (Argentina), on the Falkland Islands (Malvinas), and in northern Argentina (Bridges 1949; Rodriguez and Bastida 1998; Dans et al. 2004; Dickinson 2007), and the high $\delta^{13}C$ values of the sea lion samples recovered at the Beagle Channel from the early nineteenth century are best explained by dispersal from distant areas with a distinct isotope baseline rather than by a dietary shift of the local population.

The dramatic impact of western sealing on the diet and ecology of South American sea lions is in sharp contrast with the rather stable diet of South American sea lions during the period of aboriginal exploitation. Evidence is particularly compelling in southern Patagonia, where the zooarchaeological record spans several millennia and the stable isotope ratios in the bones of South American sea lions were rather stable. Variability in stable isotope ratios was larger in northern-central Patagonia, but considering the scarcity of samples older than 2000 ^{14}C ybp and the variability associated with the sampling of different skeletal elements, dietary shifts during the aboriginal period are uncertain.

Nevertheless, it should be noted that the diet of South American sea lions inhabiting truly pristine environments remains unknown. Most of the samples analyzed here came from archaeological sites and the oldest skeletal remains of South American sea lions from southern Patagonia are 1000 years younger than the oldest evidence of human exploitation of the marine resources in the area (Orquera and Piana 1988, 1999; Orquera et al. 2011; Tivoli and Zangrando 2011). Likewise, the oldest skeletal remains of South American sea lions from northern-central Patagonia are 3000 years younger than the oldest archaeological evidence of the exploitation of fishes, marine birds, and crustaceans in the area (Favier Dubois et al. 2009; Favier Dubois and Kokot 2011; Favier Dubois and Scartascini 2012; Gómez Otero et al. 2013). Whether such previous exploitation had modified the trophic niche of South American sea lions remains unknown and can be answered only if paleontological sites predating the arrival of humans were discovered.

In any case, the overall evidence indicates that South American sea lions currently forage at a higher trophic level than they did originally and that most of the change was related to human exploitation during the twentieth century. On the contrary, there is no evidence that aboriginal exploitation had a major effect on the trophic ecology of South American sea lions. Accordingly, the current ecology of South American sea lions is a poor guide to understanding the evolutionary forces that operated on the species throughout most of its history. For instance, there is no justification for claims that sexual differences in body mass evolved to reduce trophic overlap, as differences in the diets of male and females vanish as population size approaches carrying capacity (Drago et al. 2009, 2010). Likewise, the pelagic diet of South American sea lions prior to exploitation by western sealers (this study) suggests that the current resource partitioning between demersal South American sea lions and epipelagic South American fur seals (Franco-Trecu et al. 2012) is an artifact resulting from human exploitation, and the same might be true for other sympatric pairs where sea lion and fur seal species differentiate along a demersal-pelagic gradient (e.g., Antonelis et al. 1990; Páez-Rosas et al. 2012).

The results reported here have also implications for conservation. Preventing extinction due to human activity is the first step in wildlife conservation, but restoring the role of species in ecosystem dynamics has to be the long-term goal (Jackson and Hobbs 2009; Terborgh and Estes 2010; Bullock et al. 2011). Legal protection has certainly allowed the partial recovery of the population of South American sea lions in Argentina (Dans et al. 2004; Schiavini et al. 2004; Grandi et al. 2012), but has not restored the ecological role of the species (this study). Fishing is currently the major ecological driver of coastal ecosystems in the southwestern Atlantic (Koen-Alonso and Yodzis, 2005) and has established a new carrying capacity for South American sea lions (Drago et al. 2010). Information about the deep-time ecology of South American sea lions may assist managers in assessing whether the original niche has been restored, independently of population size. Restoring the original trophic ecology of the species is important, because only in this way will the evolutionary forces than once operated on the species also be restored.

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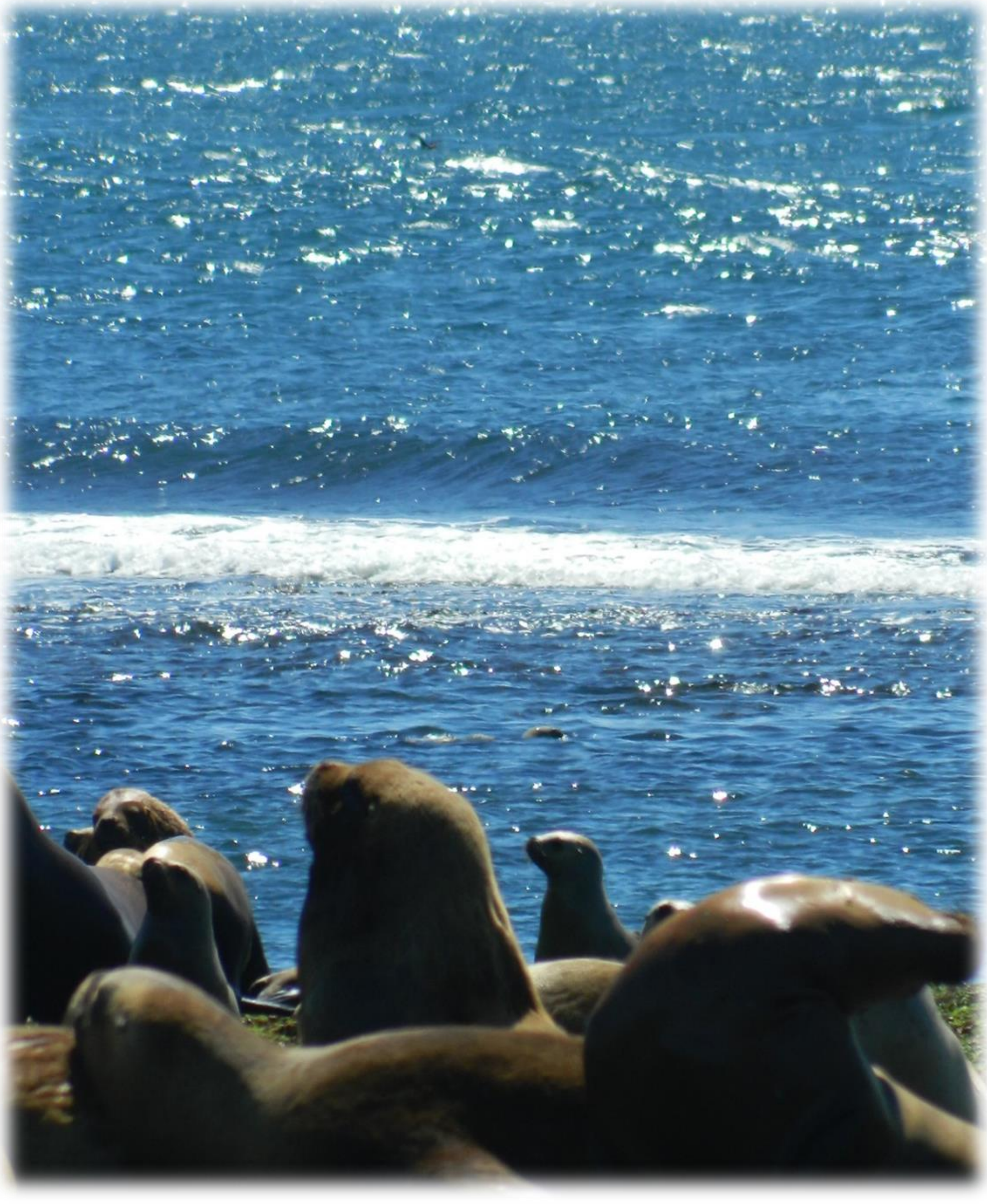
CUARTO CAPÍTULO

CAMBIOS HISTÓRICOS EN LA DIETA DE LOS LOBOS MARINOS SUDAMERICANOS



Estabilidad de la dieta del lobo común sudamericano durante tres décadas en el sur de Brasil, de acuerdo con el análisis de isótopos estables

RESUMEN. Los depredadores marinos pueden cambiar su dieta a través del tiempo como consecuencia de cambios antropogénicos y naturales. Sin embargo, esta variabilidad de la dieta es difícil de abordar y rara vez se incorpora en la modelización ecosistémica. Este trabajo utiliza las proporciones de isótopos estables de carbono y nitrógeno en el material esquelético de lobo común sudamericano procedente de colecciones científicas brasileñas, para investigar si estos animales modificaron su dieta entre los años 1986 y 2009, tal como se reportó para otros depredadores marinos en la región. Los resultados del análisis de las proporciones de isótopos estables señalaron que las presas demersales potenciales estuvieron siempre enriquecidas en ^{13}C en comparación con las presas pelágicas potenciales. Además, se observó una ausencia de correlación entre el año de varamiento y los valores $\delta^{13}C$ de los machos adultos, lo cual indica que no existió ningún aumento importante en el consumo de presas pelágicas entre 1986 y 2009. Igualmente, los resultados del modelo de mezcla SIAR revelaron para todo el período estudiado una dieta mixta formada por presas pelágicas y demersales, aunque con un papel central para los peces demersales. Además, SIAR indicó la ausencia de grandes cambios en la proporción de presas pelágicas y demersales en la dieta de los machos adultos del lobo común sudamericano durante las últimas tres décadas. Por otra parte, los peces demersales también fueron presas importantes para los lobos marinos jóvenes durante todo el periodo de estudio, aunque siempre consumieron una mayor proporción de presas pelágicas que los adultos. Se concluye que no se han producido cambios significativos en la dieta de los machos de lobo común sudamericano durante las últimas tres décadas en el sur de Brasil, en contraposición con lo sucedido con otros depredadores en la región y con la misma especie en el norte de Patagonia.

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Dietary consistency of male South American sea lions (*Otaria flavescens*) in southern Brazil during three decades inferred from stable isotope analysis

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Abstract Marine predators may undergo remarkable dietary changes through time as a result of both anthropogenic and natural changes in the environment, but this variability is often difficult to tackle and seldom incorporated into ecosystem models. This paper uses the stable isotope ratios of carbon and nitrogen in skeletal material of South American sea lions from Brazilian scientific collections to investigate whether these animals modified their diet from 1986 to 2009, as reported for other marine predators in the region. Stable isotope ratios indicated that demersal potential prey were always enriched in ^{13}C as compared with pelagic prey. Accordingly, the absence of any statistically significant correlation between stranding year and the $\delta^{13}C$ values of adult males indicated no major increase in the consumption of pelagic prey from 1986 to 2009. Likewise, the results of the mixing model SIAR revealed a mixed diet including pelagic and demersal prey, with a central role for demersal fishes throughout the whole period. Furthermore, SIAR suggested no major changes in the proportion of pelagic and demersal prey in the diet of adult male South American sea lions during the past three decades. Demersal fishes were also relevant prey for juvenile South American sea lions during the whole period, but they always consumed a larger proportion of pelagic prey than the adults did. These results suggest no major changes in the diet of male South American sea lions during the past three decades in southern Brazil, contrary to what has been reported for other to predators in the regions and for the species in northern Patagonia.

Introduction

Human activities have impacted most of the marine ecosystems around the world (Halpern 2008), and only retrospective studies can give us a full account of the magnitude of the change (Jackson et al. 2001). This approach has revealed that some marine predators have undergone remarkable dietary changes through time as a result of natural changes in food web structure (e.g., Trites et al. 2007; Páez-Rosas et al. 2012) and interaction with fisheries (e.g., Drago et al. 2009a; Hanson et al. 2009; Gómez-Campos et al. 2011). Ecosystem models need to account for those changes to produce realistic reconstructions of historical changes in ecosystem dynamics, but this is often impossible due to the absence of retrospective studies on the diet of marine predators.

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Otariids inhabiting the southeastern coast of South America were heavily exploited since the arrival of western settlers and exploitation lasted till the second half of the twentieth century (Pérez Fontana 1943; Godoy 1963; Rodríguez and Bastida 1998; Ponce de León 2000). The northernmost rookeries of the South American sea lion (*Otaria flavescens*) are found in Uruguay, where <15,000 South American sea lions were estimated to survive in 1995 and the production of sea lion pups would be descending at a rate of 4.5 % per year (Páez 2006). Conversely, the numbers of South American fur seals (*Arctocephalus australis*) breeding in the same colonies increased since the end of commercial sealing (Vaz-Ferreira 1982; Lima and Páez 1997; Franco-Trecu et al. 2012).

The reason for the differences in the post-harvest dynamics of these two species is unknown, although Costa et al. (2004, 2006) have argued that pelagic foragers recover faster than demersal ones after exploitation because pelagic resources are usually less exploited by humans than demersal ones. South American sea lions breeding in Uruguay forage over a large area spanning from southern Brazil to northern Argentina (Rodríguez et al. 2013), with high levels of individual variability in the foraging grounds used (Zenteno et al. 2013). The same region supports important demersal fisheries, but landing biomass, catch per unit effort and mean trophic level of landings declined in the mid-1990s in some areas and currently many stocks are fully exploited or overexploited (Haimovici 1998; Vasconcellos and Gasalla 2001; Jaureguizar and Milessi 2008; Milessi and Jaureguizar 2013). As a response to the above reported changes, franciscana dolphins (*Pontoporia blainvillei*) decreased the consumption of some demersal sciaenid fishes (Pinedo 1994; Secchi et al. 2003; Crespo and Hall 2002) and the overall contribution of demersal fishes to the diet of marine birds declined over the past 30 years (Bugoni 2008).

Available dietary information for the South American sea lion in the region is based on scats and stomach contents analysis and revealed no evident temporal changes off southern Brazil (Rosas, 1989; Oliveira et al. 2008; Machado, 2013) and Uruguay [Riet-Sapriza et al. (2012), but see Naya et al. (2000); Szteren et al. (2004)]. However, most of the information has been collected only recently, and scats and stomach contents are not appropriate to test long-term variation in food resources, since these methods provide only a single “snapshot” of the diet of each individual just before sampling (Iverson et al. 2004). Furthermore, repeated sampling of large animals for stomach content analysis is extremely difficult and assigning scats to particular individuals is highly unlikely in crowded rookeries (Drago et al. 2010a).

Stable isotope analysis offers an alternative method to reconstruct dietary changes in marine predators over long periods of time (e.g., Drago et al. 2009a; Hanson et al. 2009; Newsome et al. 2010a). The method is based on the assumption that 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, although stable isotope ratios experience a stepwise enrichment in the heavier isotope relative to prey (DeNiro and Epstein 1978; Kelly 2000). This increase is more pronounced in $\delta^{15}N$ values (3–5 ‰), which consequently are used to assess trophic level (DeNiro and Epstein 1978; Minagawa and Wada 1984). Trophic enrichment in ^{13}C is smaller (0.5–1.1 ‰) (Fry and Sherr 1984; Wada et al. 1991), and as a consequence, animal $\delta^{13}C$ values are useful to identify consumption of prey with different $\delta^{13}C$ values at a local scale, as well as foraging areas at larger geographic scales (Rau et al. 1982; Hobson et al. 1997).

Here, we use stable isotope ratios in skeletal material of South American sea lions available at scientific collections from Brazil to investigate whether major dietary shifts have occurred during the past three decades.

Materials and methods

Study site and sample collection

The scientific collection of Universidade Federal do Rio Grande (FURG) stores skeletal material from South American sea lions dead stranded in southern Brazil (29°S–32°S; Fig. 1) from 1986 to 1988, whereas the collection of Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS) stores skeletal material from animals dead stranded in the same area from 1994 to 2009.

Although the South American sea lion is one of the most frequently pinniped species observed off Brazil, there are no breeding colonies of the species in the area (Pinedo 1990; Simões-Lopes et al. 1995), and South American sea lions are thought to come from the breeding colonies in Uruguay, 300 km south of Rio Grande do Sul (Pinedo 1990; Rosas et al. 1994). Satellite telemetry has revealed that during the breeding season South American sea lions forage in a wide area ranging from southern Brazil to northern Argentina, but stable isotopes of oxygen have revealed limited exchange of adult male South American sea lions with other regions in the southwestern Atlantic Ocean (Zenteno et al. 2013).

Additional South American sea lion samples (bone and vibrissae) were collected from the scientific collection of Centro Nacional Patagónico (Puerto Madryn, Argentina) to calculate diet-to-predator discrimination factors (see below).

Maxillo-turbinal bones were initially selected for the present study, as sampling them preserved the collected

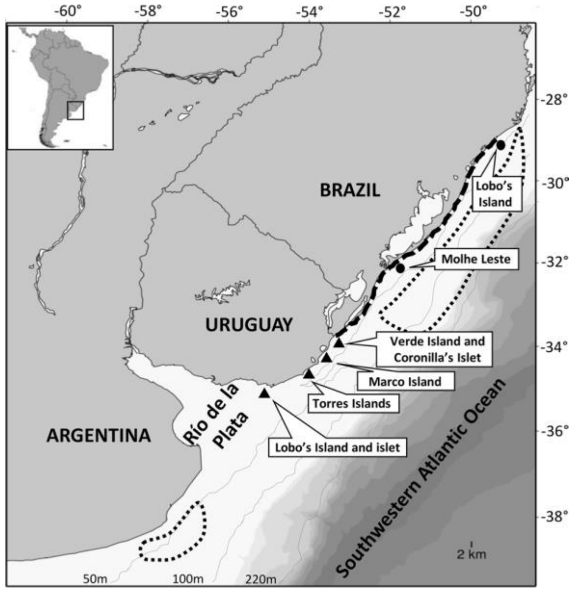

Fig. 1 Study area. South American sea lion samples were collected along the *dashed line*. Potential prey were collected along northern Argentina and southern Brazil. The *triangles* show the main breeding rookeries of South American sea lions in Uruguay, whereas the *circles* show the main haul-outs sites occupied by South American sea lions in southern Brazil. Potential prey were collected within the *dotted polygons* (*Source*: www.seaturtle.org)

skulls for further study. However, only the canine teeth of the earlier specimens had been preserved in the collection. Since stable isotope ratios of carbon and nitrogen may vary between tissues [Koch (2007), but see Riofrío-Lazo and Aurioles-Gamboa (2013)], differences in δ^{13}C and δ^{15}N values in paired samples of bone and dentine (all the layers after the second annuli) from 12 individuals were tested. Additional paired samples of vibrissae and bone from eight adult individuals were also analyzed to calculate a diet-to-bone and diet-to-dentine discrimination factors (see below).

South American sea lions may forage over a wide area including southern Brazil, Uruguay and northern Argentina (Rodríguez et al. 2013). The stable isotope ratios of some South American sea lion prey from southern Brazil, Uruguay and northern Argentina have been reported by Abreu et al. (2006), Bugoni et al. (2010), Botto et al. (2011) and Franco-Trecu et al. (2013a). Additional potential prey previously identified by stomach and scat analysis (Naya et al. 2000; Szteren et al. 2004, 2006; Suárez et al. 2005; Oliveira et al. 2008; Machado 2013) was collected. Samples were obtained from fishermen from Brazil (Santa Catarina and Rio Grande do Sul province) and northern Argentina (Buenos Aires province) in 2009 and 2010 (Fig. 1; Table 2). White dorsal muscle was sampled from fishes and mantle from cephalopods. All samples were stored in a freezer at −20 °C until analysis.

Sex and age determination

Sex was determined based on the external morphology (presence of *bacullum* bone) during sampling collection and eventually assessed according to secondary sexual characteristics of skull following Crespo (1984, 1988). Only males were considered for this study, due to the scarcity of females in the scientific collections. South American sea lions had previously been aged by counting growth layers in the dentine of the canines (assuming annual deposition) in thin ground sections or acid-etched highlighted teeth (Hohn 1980; Perrin and Myrick 1980; Crespo 1988). The life span of South American sea lions is around 20 years (Crespo 1988), and they become physiologically mature between 4 and 6 years, although mate for the first time when they are 9 years old or more (Crespo 1988; Grandi et al. 2010). Furthermore, skull growth stops at the age of 9 years (Drago et al. 2009b). Based on these data, South American sea lions 2–8 years old were considered juveniles and adolescents and those older than 8 years were considered adults. All the analysis was done independently for adults and for younger animals. Furthermore, individual age was included in the correlation analysis conducted for each age class.

Stable isotope analysis

Bone, dentine (all the layers for FURG samples) and muscle samples were thawed, dried in a stove at 60 °C for 36 h and grounded into a fine powder using a mortar and pestle. Since lipids can bias the analyses by decreasing δ^{13}C values (DeNiro and Epstein 1977), they were removed from the samples using a sequential soak in a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) and shaken with a rotator to accelerate the lipid extraction. Vibrissa was soaked in a chloroform/methanol (2:1) solution for 15 min in an ultrasonic bath. Any remaining residue on vibrissae was scrubbed off with a brush and the soaking process repeated. The samples were then dried again for 48 h at 60 °C. Vibrissae were cut into 3-mm-long consecutive sections starting from the proximal end and each section analyzed separately. This is because each section integrates diet during 1 month (Hirons et al. 2001; Cherel et al. 2009; Kernalégen et al. 2012) and the results will be used latter in a different study aiming to reconstruct monthly changes in the diet of sea lions (Zenteno, unpublished data). Here, only the average values of individual vibrissa were used, because they integrate approximately the same time span than bone (Riofrío-Lazo and Aurioles-Gamboa 2013).

Table 1 Stable isotope ratios (mean ± *SD*) in dentine and bone tissue of male South American sea lions found dead along the coast of southern Brazil

Sample number	Sex	Tissue	Death (yr)	δ^{13}C (‰)	Suess corrected δ^{13}C (‰)	δ^{15}N (‰)	Age (yr)
FURG837	♂	Tooth	1986	−10.7	−11.2	19.0	4
FURG839	♂	Tooth	1986	−12.7	−13.2	20.7	8
FURG913	♂	Tooth	1986	−11.0	−11.5	20.6	9
FURG824	♂	Tooth	1986	−11.7	−12.2	20.3	10
FURG826	♂	Tooth	1986	−11.4	−11.9	21.9	14
FURG1021	♂	Tooth	1986	−11.5	−12.0	21.8	15
FURG1016	♂	Tooth	1986	−12.5	−13.0	21.4	22
FURG1070	♂	Tooth	1987	−13.6	−14.1	20.5	5
FURG1088	♂	Tooth	1987	−13.8	−14.3	20.3	5
FURG1066	♂	Tooth	1987	−11.1	−11.6	20.9	7
FURG1063	♂	Tooth	1987	−11.6	−12.1	20.6	8
FURG1089	♂	Tooth	1987	−13.8	−14.3	20.3	8
FURG1084	♂	Tooth	1987	−10.8	−11.3	20.2	11
FURG1134	♂	Tooth	1987	−11.8	−12.3	20.4	13
FURG1202	♂	Tooth	1988	−13.7	−14.2	20.4	7
FURG1201	♂	Tooth	1988	−11.1	−11.6	21.6	14
GEMARS184	♂	Bone	1994	−12.3	−12.6	20.8	10
GEMARS193	♂	Bone	1994	−11.5	−11.8	21.2	10
GEMARS171	♂	Bone	1994	−10.6	−10.9	20.6	19
GEMARS268	♂	Bone	1995	−12.2	−12.5	20.9	5
GEMARS284	♂	Bone	1995	−12.4	−12.7	20.4	10
GEMARS288	♂	Bone	1995	−11.5	−11.8	21.2	12
GEMARS299	♂	Bone	1995	−11.9	−12.2	20.9	25
GEMARS343	♂	Bone	1996	−11.7	−12.0	20.6	4
GEMARS353	♂	Bone	1996	−12.7	−13.0	20.5	12
GEMARS358	♂	Bone	1996	−13.8	−14.1	20.0	21
GEMARS444	♂	Bone	1997	−12.2	−12.5	20.6	6
GEMARS434	♂	Bone	1997	−11.4	−11.7	20.6	8
GEMARS428	♂	Bone	1997	−11.5	−11.8	21.2	14
GEMARS517	♂	Bone	1998	−10.2	−10.4	20.0	5
GEMARS555	♂	Bone	1998	−12.7	−12.9	21.6	6
GEMARS523	♂	Bone	1998	−12.2	−12.4	21.4	10
GEMARS553	♂	Bone	1998	−11.0	−11.2	19.8	15
GEMARS516	♂	Bone	1998	−11.9	−12.1	21.2	18
GEMARS659	♂	Bone	2000	−11.1	−11.3	19.4	6
GEMARS642	♂	Bone	2000	−11.0	−11.2	20.1	10
GEMARS658	♂	Bone	2000	−11.7	−11.9	19.7	14
GEMARS667	♂	Bone	2000	−10.9	−11.1	19.1	15
GEMARS812	♂	Bone	2001	−12.4	−12.6	20.1	4
GEMARS809	♂	Bone	2001	−11.8	−12.0	20.9	12
GEMARS813	♂	Bone	2002	−13.4	−13.6	22.9	2
GEMARS868	♂	Bone	2002	−11.4	−11.6	20.1	12
GEMARS967	♂	Bone	2003	−12.4	−12.5	20.6	5
GEMARS1111	♂	Bone	2003	−12.2	−12.3	21.2	8
GEMARS1151	♂	Bone	2003	−12.1	−12.2	20.7	8
GEMARS992	♂	Bone	2003	−13.7	−13.8	20.6	11
GEMARS1040	♂	Bone	2003	−10.8	−10.9	20.4	12
GEMARS1060	♂	Bone	2003	−12.1	−12.2	20.8	12
GEMARS970	♂	Bone	2003	−11.9	−12.0	20.5	14
GEMARS1126	♂	Bone	2003	−11.4	−11.5	21.0	15

Table 1 continued

Sample number	Sex	Tissue	Death (yr)	$\delta^{13}C$ (‰)	Suess corrected $\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Age (yr)
GEMARS1189	♂	Bone	2004	−11.2	−11.3	19.8	12
GEMARS1323	♂	Bone	2009	−12.0	−12.0	20.7	9
GEMARS1303	♂	Bone	2008	−12.2	−12.2	20.3	15
GEMARS1345	♂	Bone	2009	−11.9	−11.9	20.9	11

Ratios corrected for the Suess effect are referenced to 2009

As bones and teeth 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 decarbonise them (Ogawa and Ogura 1997). Since acidification may modify $\delta^{15}N$ values (Bunn et al. 1995), samples were divided into two subsamples, one used to measure $\delta^{13}C$ values following acidification and the other to measure $\delta^{15}N$ values prior to acidification.

Approximately 0.3 mg of vibrissae, 0.4 mg of dentine, 0.8 mg of bone and 0.3 mg of white muscle from fish and mantle from cephalopods were weighed into tin capsules (3.3 × 5 mm), combusted at 900 °C 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 Centres Cientifics i Tecnològics de la Universitat de Barcelona.

The abundances of stable isotopes, expressed in delta (δ) notation, were the relative variations of stable isotope ratios expressed as per thousand (‰) deviations from predefined international standards as:

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N, and R_{sample} and R_{standard} are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the sample and standard, respectively. The $\delta^{13}C$ standard was Vienna PeeDee Belemnite (VPDB) calcium carbonate, and $\delta^{15}N$ standard was atmospheric nitrogen (N_2). International standards (ammonium sulfate, potassium nitrate, glutamic acid for $\delta^{15}N$ and polyethylene, sucrose and glutamic acid for $\delta^{13}C$) were inserted after every 12 samples to calibrate the system and compensate for any drift over time. Precision and accuracy for $\delta^{13}C$ and $\delta^{15}N$ measurements were 0.1 and 0.3 ‰, respectively.

Suess effect correction

The content of ^{13}C in atmospheric CO_2 has decreased 0.022 per mil/year since 1960, due largely to fossil fuel burning (Francey et al. 1999; Indermühle et al. 1999). For that reason, we have corrected the original $\delta^{13}C$ values of the skeletal material shown in Table 1 to account for such a decrease and allow comparison among samples from different periods. All the corrected $\delta^{13}C$ values were referenced to 2009.

Stable isotope discrimination factors

The use of appropriate diet–tissue discrimination factors is one of the most important basic requirements when applying stable isotope mixing models to predict the dietary sources of a consumer and the trophic position relative to primary consumers (Newsome et al. 2010a). In pinnipeds, previous studies have assessed discriminating factors between diet and blood, skin and vibrissae (Hobson et al. 1996), but nothing is known about the diet-to-bone discrimination factor. Here, we calculated two discrimination factors using different approaches.

The first discrimination factor was calculated using previously published information about diet composition from northern Patagonia (Koen-Alonso et al. 2000), stable isotope ratios of potential prey from that area (Drago et al. 2010b) and stable isotope ratios in the bone of South American sea lions from the same area (Drago et al. 2009a). The second discrimination factor was calculated using previously published information about diet-to-vibrissa discrimination in marine mammals (Hobson et al. 1996; Newsome et al. 2010b) and the stable isotope ratios in paired samples of vibrissa and skull from the CENPAT scientific collection. This latter diet-to-bone fractionation was computed as follows:

$$\Delta_{(\text{bone - vibrissae})} + \Delta_{\text{Means Reference (vibrissae - diet)}} = \Delta_{(\text{bone – diet})} \qquad (2)$$

Data analysis

Data are presented as mean ± standard deviation (SD), and significance was assumed at the 0.05 level. All statistical analyses were carried out with PASW Statistics (version 17.0 for Windows, SPSS). As long as the assumptions of normality (tested using Lilliefors's test) and homoscedasticity (tested using Levene's test) were met, parametric approaches (Pearson's correlation and ANCOVA) were used.

Two-way ANOVA was used to compare the stable isotope ratios of potential prey in southern Brazil and northern Argentina. Potential prey from Uruguay was not included in the analysis because only average and standard deviation values have been published (Franco-Trecu et al. 2013a). Temporal trends in the isotopic signal of the bones and teeth of South American sea lion were investigated

Table 2 Stable isotope ratios (mean ± *SD*) of the potential prey species for South American sea lions off southern Brazil and northern Argentina

Sample			*n*	δ^{13}C (‰) (Mean ± SD)	δ^{15}N (‰) (Mean ± SD)
Southern Brazil					
Medium pelagic fishes					
Cynoscion guatucupa	Stripped weakfish	Sciaenidae	5	−16.5 ± 0.7	16.0 ± 0.6
Macrodon atricauda	King weakfish	Sciaenidae	5	−14.5 ± 0.7	17.0 ± 0.3
Pomatomus saltatrix	Bluefish	Pomatomidae	5	−16.6 ± 0.5	18.6 ± 0.7
Trachurus lathami[a]	Rough scad	Carangidae	9	−16.1 ± 0.1	16.1 ± 1.1
Trichiurus lepturus	Cutlassfish	Trichiuridae	5	−15.7 ± 0.3	16.6 ± 0.5
Small Pelagic Fishes					
Engraulis anchoita[a]	Argentine anchovy	Engraulidae	14	−16.5 ± 0.5	14.7 ± 0.5
Sardinella brasiliensis[a]	Brazilian sardine	Lupeidae	7	−17.6 ± 0.6	11.4 ± 1.0
Demersal Fishes					
Genypterus brasiliensis[a]	Pink cusk-eel	Ophidiidae	1	−16.1	14.7
Menticirrhus americanus	Southern kingfish	Sciaenidae	5	−15.4 ± 0.5	−16.0 ± 0.5
Micropogonias furnieri	White croacker	Sciaenidae	5	−15.7 ± 0.6	15.3 ± 0.4
Paralonchurus brasiliensis[c]	Banded croacker	Sciaenidae	5	−14.2 ± 0.5	15.6 ± 0.6
Polyprion americanus[a]	Wreckfish	Polyprionidae	1	−15.7	17.7
Prionotus nudigula[a]	Bluewing searobin	Triglidae	9	−15.0 ± 0.5	16.9 ± 0.7
Sparus pagrus[a]	Red porgy	Sparidae	1	−15.9	15.5
Umbrina canosai[a]	Argentine croaker	Sciaenidae	10	−15.5 ± 0.3	16.6 ± 0.8
Urophycis brasiliensis[a]	Brazilian codling	Phycidae	11	−15.6 ± 0.5	16.5 ± 0.7
Demersal Pelagic Cephalopods					
Illex argentinus	Argentine short-finned squid	Ommastrephidae	5	−18.1 ± 0.2	10.0 ± 0.5
Loligo paelei	Atlantic longfin squid	Loliginidae	5	−17.6 ± 0.2	11.3 ± 0.5
Northern Argentina					
Medium Pelagic Fishes					
Cynoscion guatucupa	Striped weakfish	Sciaenidae	5	−17.2 ± 0.2	17.6 ± 0.3
Merluccius hubbsi (>30 cm)	Argentine hake	Merlucciidae	3	−18.0 ± 0.5	15.6 ± 0.4
Pseudopercis semifasciata	Argentine sandperch	Pinguipedidae	4	−16.5 ± 0.3	18.2 ± 0.4
Trachurus picturatus	Blue jack mackerel	Carangidae	5	−17.6 ± 0.3	16.9 ± 0.5
Small Pelagic Fishes					
Engraulis anchoita	Argentine anchovy	Engraulidae	5	−18.2 ± 0.3	15.5 ± 0.5
Merluccius hubbsi (<30 cm)	Argentine hake	Merlucciidae	2	−18.4 ± 0.1	14.8 ± 0.0
Sorgentinia incisa	Silverside	Atherinopsidae	5	−17.6 ± 0.3	15.5 ± 0.2
Stromateus brasiliensis	Butterfish	Stromateidae	5	−16.9 ± 0.6	17.4 ± 0.4
Demersal Fishes					
Menticirrhus americanus	Southern kingfish	Sciaenidae	5	−15.0 ± 0.9	19.1 ± 1.1
Micropogonias furnieri	White croacker	Sciaenidae	5	−16.0 ± 0.2	16.7 ± 0.5
Prionotus nudigula	Bluewing searobin	Triglidae	5	−17.0 ± 0.1	18.0 ± 0.6
Paralichthys sp	Flounder	Paralichthyidae	5	−16.7 ± 0.2	17.7 ± 0.2
Raneya brasiliensis	*Banded cusk-eel*	Ophidiidae	5	−16.2 ± 0.4	17.4 ± 0.6
Sparus pagrus	Red porgy	Sparidae	6	−16.3 ± 0.6	17.4 ± 0.5
Demersal Pelagic Cephalopods					
Illex argentinus	Argentine short-finned squid	Ommastrephidae	5	−17.5 ± 0.4	14.7 ± 0.5

using partial correlation coefficients controlling for ages Although bone and dentine integrate dietary information over long periods, stranding year was used as a temporal reference, without any attempt to calculate the central year of the time span integrated by each individual. δ^{13}C values were corrected for the Suess effect.

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[a] Reference: Bugoni et al. (2010); [b] Reference: Franco-Trecu et al. (2013a); [c] Reference: Silva-Costa and Bugoni (2013)

Finally, SIAR, a Bayesian mixing model Stable Isotope Analysis in R (Parnell et al. 2010) package for software R (R Development Core Team 2009), was used to assess the relative contributions of potential prey species to the diet of South American sea lion males dead stranded before 1990 and after 1999. There were two reasons for that partitioning. First, only dentine samples were available before 1989 and only bone samples were available since 1994 (Table 1). Second, fisheries operating in the adjoining Argentinean–Uruguayan Common Fishing Zone suffered major changes in the average trophic level of landings during the mid-1990s (Jaureguizar and Milessi 2008; Milessi and Jeureguizar 2013). Although the significance of those changes for the availability of potential prey for South American sea lions in southern Brazil remains unknown, the exclusion from the analysis of those specimens that lived during that period aims to control such a possible influence.

SIAR estimates the probability distributions of multiple source contributions to a mixture while accounting for the observed variability in source and mixture isotopic compositions, dietary isotopic fractionation and elemental concentration. The model included prey species that were clumped into ecological groups: All the demersal fishes together, small pelagic fishes from Brazil, small pelagic fishes from Argentina, demersal pelagic cephalopods from Brazil and demersal pelagic cephalopods from Argentina. The species included in each group were selected according to previous studies analyzing stomach contents and scats from the region (Oliveira et al. 2008; Machado 2013; Naya et al. 2000; Szteren et al. 2004; Riet-Sapriza et al. 2012; Suárez et al. 2005), although they may not give full coverage of the diet due to seasonal biases in sampling. Data within each group fitted a normal distribution, as this is required by SIAR (Parnell et al. 2010). The model was run twice, using the two sets of fractionation factors obtained in this study.

Results

The stable isotope ratios of potential prey from northern Argentina and southern Brazil are shown in Table 2. Potential prey from northern Argentina was usually depleted in ^{13}C and enriched in ^{15}N when compared with the same species from southern Brazil (two-way ANOVA; δ^{13}C: $F_{(11,\ 48)} = 37.41$, $P < 0.001$; δ^{15}N: $F_{(11,48)} = 32.15$, $P < 0.001$). However, the species–area interaction term was statistically significant in both cases (δ^{13}C $F_{(11,\ 48)} = 8.12$, $P < 0.001$; δ^{15}N: $F_{(11,\ 48)} = 12.3$, $P < 0.001$), thus indicating that some species departed from that pattern. Nevertheless, demersal fishes from the two regions were more enriched in ^{13}C than any other group of potential prey and small pelagic fishes from both regions were more depleted in ^{15}N that any other group (Fig. 2). For further analysis, prey was pooled into ecological groups differing in average stable isotope ratios: demersal fishes, medium-size pelagic fishes, small pelagic fishes from Brazil, small pelagic fishes from Argentina, demersal pelagic cephalopods from Brazil and demersal pelagic cephalopods from Argentina.

Paired samples of bone and dentine from adult South American sea lions did not differ in average δ^{13}C values ($\delta^{13}C_{\text{bone}} = -11.9 \pm 0.4$ ‰; $\delta^{13}C_{\text{dentine}} = -12.0 \pm 0.5$ ‰; paired t test; $t = 0.571$, $P = 0.574$, $n = 12$ for each tissue), but dentine was depleted in ^{15}N when compared with bone from the same individual ($\delta^{15}N_{\text{bone}} = 22.2 \pm 0.8$ ‰, $\delta^{15}N_{\text{dentine}} = 21.4 \pm 0.6$ ‰; paired t test; $t = 2.763$, $P = 0.011$, $n = 12$ for each tissue). Accordingly, only the δ^{13}C values from the whole data set can be considered to analyze temporal changes while analysis of δ^{15}N values had to be limited to the 1994–2009 period (bone samples).

When the whole data set of males South American sea lions older than 9 years was considered (years 1986–2009), stranding year and δ^{13}C values were uncorrelated (Fig. 3a; δ^{13}C: partial correlation, $r = 0.0.038$, $N = 34$, $P = 0.834$) and the same was true for the juvenile and adolescent males (Fig. 3b; δ^{13}C: partial correlation, $r = 0.332$, $N = 20$, $P = 0.165$). This result is unlikely to be an artifact of combining dentine and bone δ^{13}C values, not only because the absence of statistically significant differences above reported, but also because the variability of the δ^{13}C values was similar in the three decades (Table 3). The coefficient of variation was always <10 % of the mean, and δ^{15}N values were also uncorrelated when only the bone data set (1994–2009) was considered, both for adult males more than 9 years old (Fig. 3c; δ^{15}N: partial correlation, $r = -0.201$, $N = 26$, $P = 0.336$) and juvenile males <8 years old (Fig. 3d; δ^{15}N: partial correlation, $r = 0.219$, $N = 12$, $P = 0.519$). The variability of the δ^{15}N values was similar in the three decades (Table 3). The coefficient of variation was always <10 % of the mean.

The expected stable isotope ratios of the diet of South American sea lions from Northern Patagonia were $\delta^{13}C = -16.6$ ‰ and $\delta^{15}N = 17.0$ ‰ (Table 4), and the stable isotope ratios of male South American sea lions bone from the same area were $\delta^{13}C = -12.2 \pm 0.8$ ‰ and $\delta^{15}N = 22.3 \pm 1.3$ ‰. This resulted into a diet-to-bone discrimination factor of 4.4 ± 0.8 ‰ for δ^{13}C and 5.3 ± 1.3 ‰ for δ^{15}N. Vibrissae of South American sea lions from northern Patagonia were depleted both in ^{13}C and ^{15}N relative to bone (mean δ^{13}C: vibrissae $= -13.1 \pm 0.8$; bone $= -12.3 \pm 0.8$; mean δ^{15}N: vibrissae $= 21.2 \pm 0.9$; bone $= 22.5 \pm 1.5$), which resulted into a diet-to-bone discrimination factor of 3.5 ± 0.8 ‰ for δ^{13}C and 4.4 ± 0.8 ‰ for δ^{15}N when combined with the published diet-to-vibrissa discrimination factors.

Fig. 2 Bivariated stable isotope ratios of prey and South American sea lion males from southern Brazil after correcting them with the indirect vibrissa–bone discrimination factor (**a**) and the direct discrimination factor (**b**) and their main prey from southern Brazil and northern Argentina. Bone and dentine samples are denoted by *circles* and *triangles*, respectively. *Open symbols* represent adult South American sea lions older than 9 years, and *solid symbols* represent adult South American sea lions younger than 8 years

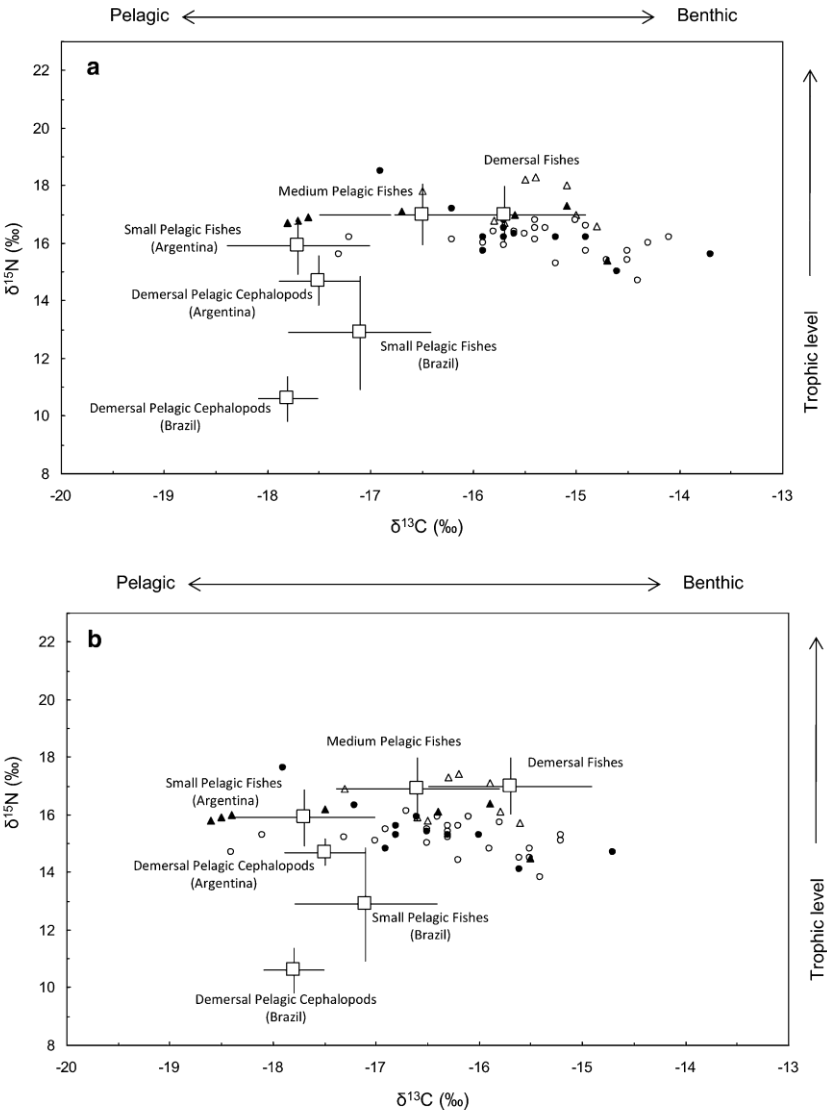

Figure 2 shows the position of potential prey and South American sea lions within the regional isoscape once the stable isotope ratios of the predator have been corrected for the Suess effect and diet-to-predator stable isotope discrimination. Most of the South American sea lion samples, independently on the tissue, were close to demersal prey when the indirect vibrissa–bone discrimination factor was used, although a few South American sea lion samples had stable isotope ratios consistent with pelagic foraging (Fig. 2a; Table 5). Conversely, the stable isotope ratios of South American sea lion samples were intermediate between those of demersal and medium-size pelagic prey when the direct prey–bone discrimination factor was used, thus suggesting more mixed diets (Fig. 2b; Table 5).

The output of SIAR confirmed that demersal and medium-size pelagic fishes dominated the diet of South American sea lions older than 9 years during the whole considered period, although the actual proportions varied according to the discrimination factor used and the importance of medium-size pelagic fishes might have increased slightly after 1994 (Figs. 4, 5; Table 5). On the other hand, pelagic prey was always more relevant for the diet of juveniles males younger than 8 years than for adults, and no major dietary shift was observed during the period considered, although the actual proportion of pelagic and

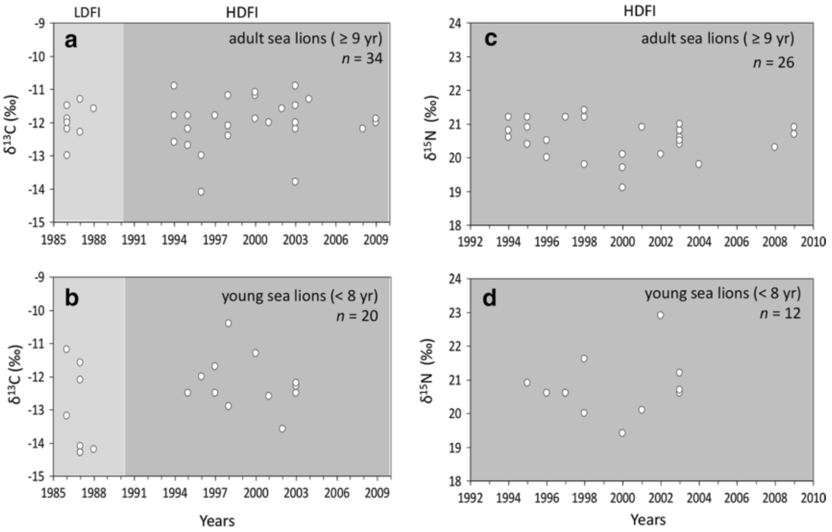

Fig. 3 Temporal changes in the ratios of stable isotopes of carbon and nitrogen in South American sea lions dead stranded along the coast of southern Brazil. The *lighter area* represents the period of low demersal fishing intensity (LDFI-years 1975–1989) and the *darker area* represents the period of increasing demersal fishing intensity (HDFI-years 1990–2010), accordingly by Haimovici (1998) and Milessi and Jaureguizar (2013). *Left panels* presents bone and tooth dentine $\delta^{13}C$ values from specimens older than 9 years (**a**) and younger than 8 years (**b**) stranded between 1986 and 2009. *Right panels* present bone $\delta^{15}N$ values from specimens older than 9 years (**c**) and younger than 8 years (**d**) stranded between 1992 and 2009. The $\delta^{13}C$ values were corrected for the Suess effect. See Table 2 for the original data

Table 3 Mean and standard deviation of the stable isotope ratios (mean ± *SD*) of male South American sea lions from southern Brazil at three different periods

	1986–1988	1994–1998	2000–2009
$\delta^{13}C$ (‰)			
<9 years old	−12.6 ± 1.3	−11.7 ± 0.8	−12.3 ± 0.7
≥9 years old	−11.5 ± 0.5	−11.9 ± 0.9	−11.7 ± 0.7
$\delta^{15}N$ (‰)			
<9 years old	20.3 ± 0.6	20.7 ± 0.5	20.8 ± 1.9
≥9 years old	21.0 ± 0.7	20.8 ± 0.5	20.4 ± 0.5

Canine dentine was analyzed in 1986–1988 and bone hereafter

demersal prey depended on the fractionation factor used (Figs. 4b, d and 5b, d).

Discussion

South American sea lions have been reported as broad-spectrum predators (Aguayo and Maturana 1973; Koen-Alonso et al. 2000; Naya et al. 2000) and diet often overlaps, at least partially, with fisheries catch in most of their range (Koen-Alonso et al. 2000; Hückstädt and Antezana 2003; Oliveira et al. 2008; Romero et al. 2011; Riet-Sapriza et al. 2012; Machado 2013). Nevertheless, they are often considered to have a low vulnerability to the development of demersal fisheries because of a high trophic plasticity (Koen-Alonso et al. 2000; Müller 2004; Szteren et al. 2004). The data presented here confirm that adult male South American sea lions from southern Brazil had mixed demersal/pelagic diets through the study period and hence suggest that no major dietary changes happened since the 1980s.

Nevertheless, historical changes in the isotopic baseline may hinder the interpretation of retrospective studies on trophic level and food web structure (Casey and Post 2011), and thus, the interpretation of isotopic signals without relevant ecological data can be challenging. In this study, $\delta^{13}C$ values were corrected to account for the Suess effect (Francey et al. 1999; Indermühle et al. 1999), but reference samples from historical fish and invertebrates were not available, and hence, other sources of variation were not controlled. For instance, an increase in the arrival of sewage during the last decades might have enhanced primary productivity and simultaneously increased the $\delta^{15}N$ values of the coastal food web (Calvert et al. 1992; Wu et al. 1997). However, available evidence revealed no major changes in primary productivity in southern Brazil from 1998 to 2006 (Heileman and Gasalla 2008), and accordingly, no major change in the $\delta^{15}N$ baseline is expected, as both parameters are strongly correlated along the coasts of the southwestern Atlantic (Saporiti et al. 2014). In any

Table 4 Data used to compute the discrimination factor on the basis of published dietary information and bone stable isotope ratios of male South American sea lions off northern Patagonia, Argentina

Potential prey	Contribution to diet[a] (%)	Stable isotope ratios			Protein contents[b] (% wet mass)		Weighted factors
		n	δ^{13}C (‰)	δ^{15}N (‰)	n		
Engraulis anchoita	0.9	5	−17.9 ± 0.2	15.7 ± 0.8	5	13.5 ± 1.2	0.01
Genypterus blacodes	3.7	–	−15.4	18.0	–	12.5	0.04
Merluccius hubbsi	56.0	5	−17.7 ± 0.6	15.9 ± 0.5	5	12.5 ± 0.5	0.57
Paralichthys isósceles	2.8	5	−15.9 ± 0.4	18.0 ± 0.6	5	13.5 ± 1.4	0.03
Pseudopercis semifasciata	12.3	–	−14.7	19.5	–	12.5	0.12
Raneya brasiliensis	3.0	5	−15.3 ± 0.6	18.8 ± 0.5	2	13.5 ± 0.2	0.03
Enteroctopus megalocyathus	10.0	5	−14.6 ± 0.7	18.9 ± 0.9	2	11.1 ± 1.6	0.09
Octopus tehuelchus	0.4	5	−14.8 ± 0.2	19.9 ± 0.4	5	12.0 ± 0.4	0.00
Illex argentinus	8.8	5	−17.0 ± 0.6	17.7 ± 0.8	5	11.0 ± 1.1	0.08
Loligo gahi	2.0	4	−17.6 ± 0.4	15.7 ± 0.6	4	13.1 ± 0.4	0.02

[a] Koen-Alonso et al. (2000); [b] Drago et al. (2010b)

Table 5 Relative contribution of demersal and pelagic prey to the diet of male South American sea lions

Potential contributions	Discrimination factor			
	Indirect vibrissae–bone	Direct	Indirect vibrissae–bone	Direct
	Dentine/adult South American sea lions (≥9 years) 1986–1988		Bone/adult South American sea lions (≥9 years) 1994–2009	
Demersal fishes	0.56	0.37	0.42	0.29
Medium pelagic fishes	0.23	0.28	0.23	0.19
Small pelagic fishes (Brazil)	0.04	0.07	0.08	0.14
Small pelagic fishes (Argentina)	0.08	0.14	0.11	0.11
Demersal pelagic cephalopods (Brazil)	0.03	0.04	0.05	0.13
Demersal pelagic cephalopods (Argentina)	0.06	0.11	0.12	0.14
	Dentine/young South American (<8 years) 1986–1988	sea lions	Bone/young South American sea (<8 years) 1994–2009	lions
Demersal fishes	0.33	0.16	0.35	0.17
Medium pelagic fishes	0.31	0.17	0.24	0.16
Small pelagic fishes (Brazil)	0.05	0.10	0.10	0.13
Small pelagic fishes (Argentina)	0.18	0.18	0.13	0.21
Demersal pelagic cephalopods (Brazil)	0.03	0.13	0.06	0.14
Demersal pelagic cephalopods (Argentina)	0.09	0.27	0.11	0.20

Results of SIAR mixing models applied with different discrimination factors, showing the mean proportion (%) of each probable source in diet of male South American sea lions

case, access to historical samples of potential prey will be extremely useful to be completely rule out changes in the stable isotope baseline during the period considered.

A second limiting factor is the existence of two tissues integrating dietary information over different time spans. Pinniped bone has been claimed to integrate dietary information throughout ~5 years, whereas canine dentine integrates dietary information through life (Riofrío-Lazo and Aurioles-Gamboa 2013). The difference is because bone is metabolically active and undergoes constant turnover, whereas dentine is metabolically inert and new layers are settled throughout the life of the individual into the open pulp cavity of the canine teeth (Riofrío-Lazo and Aurioles-Gambioa 2013). However, the actual significance of these differences for diet reconstruction is probably limited to young individuals. 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 (Ducatez et al. 2008; Drago et al. 2009b; Newsome et al. 2010a). The suckling

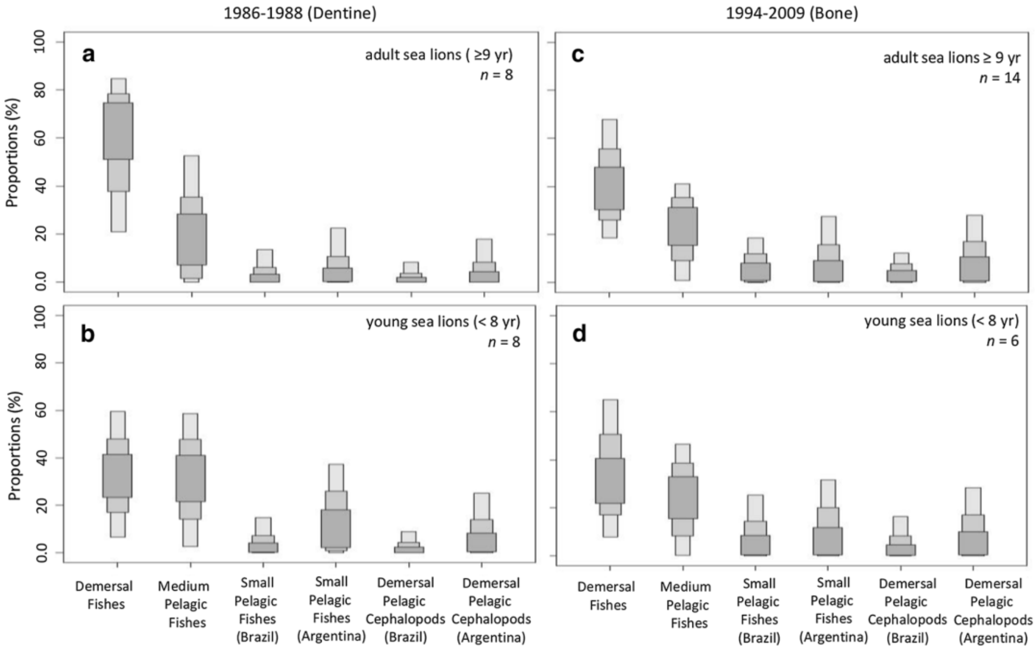

Fig. 4 Diet composition of male South American sea lions off southern Brazil according to SIAR mixing model and the indirect vibrissa–bone discrimination factor. The contribution of each prey to the diet is shown with 95, 75 and 50 % credibility intervals. The δ^{13}C values of South American sea lions were corrected for the Suess effect, to allow comparison with modern preys. See Table 2 for the original data

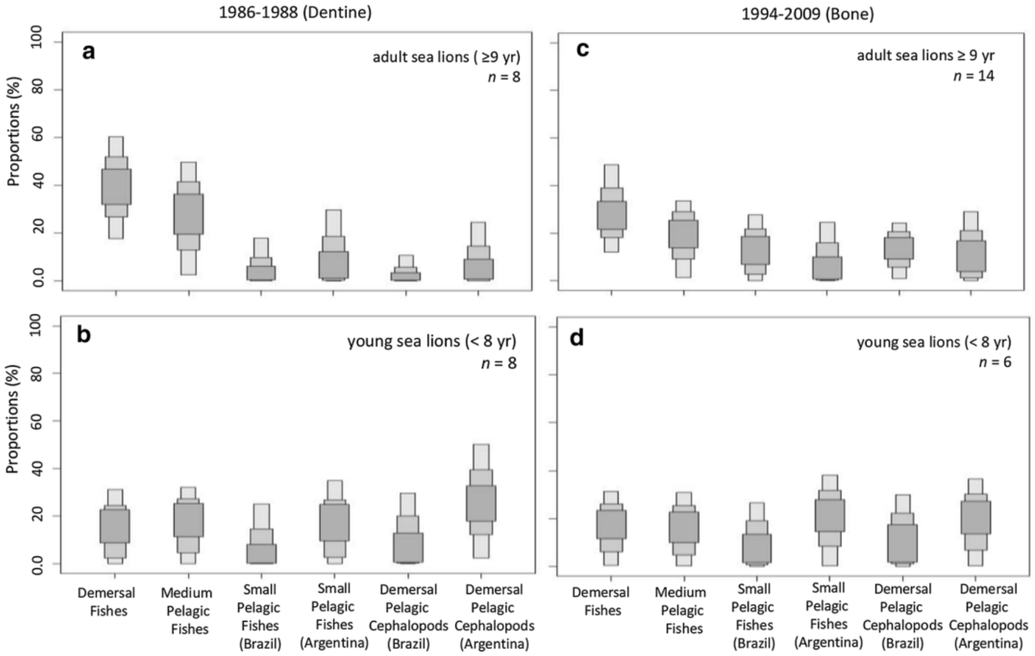

Fig. 5 Diet composition of male South American sea lions off southern Brazil according to SIAR mixing model and the direct bone discrimination factor. The contribution of each prey to the diet is shown with 95, 75 and 50 % credibility intervals. The δ^{13}C values of South American sea lions were corrected for the Suess effect, to allow comparison with modern preys. See Table 2 for the original data

signal remains forever in the dentine formed during the first year of life, but fades from bone after 1 or 2 years due to tissue turnover (Drago et al. 2009b; Newsome et al. 2010a). Accordingly, the dietary reconstructions using dentine and bone from individuals older than 2 years may lead to different conclusions about trophic level. However, the impact of the suckling signal on the overall stable isotope ratio of dentine decreases as new layers are settled and is expected to have a negligible impact on adults, where represents <1/9 of dentine.

Independently of these obscuring factors, the results here reported reveal a remarkable dietary stability of both adults and juveniles during 30 years, although there is a high level of individual variability during the whole time span of the study, independently on the age class and tissue considered. There are at least two possible caused for such variability. First, South American sea lions forage over a wide area including southern Brazil, Uruguay and northern Argentina (Rodríguez et al. 2013), and prey from those regions is known to differ in their stable isotope ratios (Abreu et al. 2006; Bugoni et al. 2010; Botto et al. 2011; Franco-Trecu et al. 2013a; this study). We are uncertain about the actual foraging area used by each individual and for how long they foraged off southern Brazil, but stable isotopes of oxygen suggest some individual differences in the foraging grounds used (Zenteno et al. 2013). Second, the existence of different individual foraging strategies cannot be excluded, as the stable isotope ratios of some adult males are closer to those of midsize pelagic fishes than to those of demersal ones. Nevertheless, stomach content analysis (Oliveira et al. 2008; Machado 2013) and stable isotope analysis (this study) agree in identifying demersal fishes as the staple food of South American sea lions in southern Brazil. Scat analysis indicates that females breeding in Uruguay also forage primarily on demersal fishes, at least during the breeding season (Riet-Sapriza et al. 2012).

There are at least two non-excluding explanations for the intense use of demersal prey by adult South American sea lions, despite of the high abundance of pelagic prey in the study area. Firstly, a selection based on prey size, as benthic prey is usually larger than pelagic prey. Secondly, a preference for benthic prey would be explained by their more sedentary behavior (Womble and Sigler 2006) and the permanent motion of pelagic prey (Gende and Sigler 2006). The first hypothesis is supported by the larger size of the demersal prey consumed by South American sea lions when compared with that of pelagic prey (Szteren et al. 2004; Riet-Sapriza et al. 2012), although pelagic prey has a higher energy density (Drago et al. 2009a).

Demersal fishes also had a central role in the diet of juvenile and adolescent males, but small and medium pelagic fishes represented the bulk of their diet. Ontogenic dietary changes in pinnipeds are often related to somatic growth and the associated improvement in diving performance (Gentry et al. 1986; Horning and Trillmich 1997; Costa et al. 2004). South American sea lions are not an exception, and they dive deeper (Rodríguez et al. 2013) and increase the consumption of demersal prey as they grow older (Drago et al. 2009b). This was also the pattern observed in the present study and suggests that the scats from unknown individuals with a high proportion of small pelagic fish (Naya et al. 2000; Szteren et al. 2004) likely represent the diet of juvenile and adolescent South American sea lions.

The dietary stability of the South American sea lion *Otaria flavescens* in southern Brazil is opposite to the dietary changes reported from northern and central Patagonia, where South American sea lions have increased the consumption of pelagic prey since the 1970 (Koen-Alonso et al. 2000; Drago et al. 2009a; Romero et al. 2011), in parallel to the development of the bottom trawling fishery but also to the increase in the South American sea lion population resulting from legal protection (Drago et al. 2009a). On the contrary, the population of the South American sea lion is decreasing in Brazil, Uruguay and northern Argentina (Páez 2006). This suggests that the per capita availability of demersal prey for the South American sea lion may have declined in northern Patagonia but remained stable in southern Brazil during the last three decades, which may explain why diet changed dramatically in the former (Drago et al. 2009a) but remained stable in the latter (this study). On the contrary, franciscana dolphins and sea birds from northern Argentina and southern Brazil have shifted diets during the past three decades (Pinedo 1994; Secchi et al. 2003; Crespo and Hall 2002; Bugoni 2008), which suggest species-specific responses to environmental changes, probably linked to differences in body size and diving performance (Páez-Rosas et al. 2012).

In conclusion, the results reported here do not support a major dietary shift for male South American sea lions during the past three decades in southern Brazil, opposite to the pattern reported in other top predators in the region which may be related to differences in body size and population dynamics (Drago et al. 2011). Certainly, females have not been considered in this study, but recent published information based on scat analysis and stable isotopes suggests a diet very similar to that of males (Riet-Sapriza et al. 2012; Franco-Trecu et al. 2013b).

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DISCUSIÓN GENERAL

“En cuestiones de ciencia, la autoridad de miles no vale más
que el humilde razonamiento de un único individuo”
Galileo Galilei

El objetivo general de esta tesis doctoral consistió en analizar los efectos de la explotación humana y los efectos del desarrollo de la industria pesquera sobre la dieta del lobo marino sudamericano en el Atlántico sur, y relacionar los eventuales cambios en la dieta con cambios en las abundancias poblacionales. Como se mencionó en la hipótesis inicial, se esperó que esta especie muestre un cambio en la posición trófica asociado a la caza comercial por parte de los colonizadores europeos, debido a la disminución de la competencia intraespecífica (Drago et al. 2009b). En cambio, no se esperaba que la caza aborigen tuviera un impacto relevante sobre la población y la dieta del lobo común.

En primer lugar se analizaron los patrones de dispersión de los lobos marinos sudamericanos (primer capítulo), con el fin de evaluar si se dispersaban habitualmente entre regiones del Atlántico sur con diferentes características isotópicas (Saporiti et al. 2015), pues de ello dependía la forma de agrupar las muestras para estudios posteriores. Con los resultados de los análisis de isótopos estables de oxígeno en la apatita del hueso de machos adultos se puso de manifiesto un intercambio limitado de lobos marinos sudamericanos entre Brasil-Uruguay, norte de Patagonia y Tierra del Fuego en Argentina, por lo que existirían al menos tres unidades de estudio en el Atlántico sur.

En el segundo capítulo se evaluó la relevancia de la dieta como único factor de variabilidad en la señal isotópica de las vibrisas de lobo marino sudamericano. Se pudo comprobar que si bien los cambios de dieta tienen un efecto evidente sobre la señal isotópica en los animales silvestres, los cambios fisiológicos, especialmente los asociados al ayuno prolongado, juegan también un papel relevante a la hora de determinar la variabilidad en los valores de $\delta^{13}C$ y $\delta^{15}N$ de los tejidos inertes de machos adultos.

Luego, sólo tras aclarar esta dos cuestiones, ha sido posible abordar los cambios de posición trófica desde finales del Holoceno hasta el presente de los lobos marinos sudamericanos (tercer capítulo), utilizando el hueso como material de referencia. Los resultados revelan que esta especie actualmente se alimenta en un nivel trófico más elevado que durante el Holoceno tardío, habiendo identificado el siglo XX como el período en el que se produjo la mayor parte del cambio del nivel trófico tras la eliminación masiva de individuos por parte de los colonizadores europeos. Por el contrario, durante el periodo de la explotación aborigen no se detectó ningún efecto importante sobre la ecología trófica de estos animales. Por otra parte, tampoco se observaron cambios significativos en la dieta de los lobos marinos sudamericanos durante las últimas tres décadas en el sur de Brasil (cuarto capítulo), a pesar del intenso desarrollo de la industria pesquera del Río de la Plata y zonas adyacentes a partir de 1990.

La conectividad entre las poblaciones de lobo marino sudamericano

El estudio de los patrones de dispersión de los lobos marinos sudamericanos mediante el análisis de los isótopos estables de oxígeno, mostró que los machos adultos son bastante fieles a una región costera en particular, al menos durante varios años, Los estudios previos, realizados mediante marcas externas, habían indicado que tras la temporada reproductiva, los machos adultos podían dispersarse a mayores distancias que las hembras (Vaz-Ferreira 1982; Rosas et al. 1994; Giardino et al. 2009, 2014), reportándose movimientos individuales entre 400-800 km (Giardino et al. 2014). Ahora bien, no todos los machos necesariamente se dispersarían, ya que algunos especímenes han mostrado un alto grado de fidelidad a ciertos lugares durante la temporada no reproductiva (Giardino et al. 2009, 2014). Lamentablemente, las marcas externas no permanecen mucho tiempo sobre el cuerpo de los lobos marinos sudamericanos (Oliveira 2010), por lo que escasean los reavistamientos a los pocos meses de ser marcados (Giardino et al. 2009, 2014). Del mismo modo, los dispositivos satelitales también permanecen unidos a estos animales por solo unos pocos meses y no dan información sobre los movimientos interanuales (Campagna et al. 2001; Riet-Sapriza et al. 2013). Como consecuencia de esto, la proporción de machos adultos que realmente se desplazan a zonas de alimentación muy alejadas tras la temporada reproductiva sigue siendo desconocida.

El análisis de isótopos estables ofrece un enfoque alternativo para la comprensión de los patrones de dispersión en mamíferos marinos, pero antes es necesario evaluar si los sesgos de edad, el origen de las muestras y los comportamientos reproductivos afectan a la variabilidad de los valores de $\delta^{18}O$ en los tejidos de estos animales. En este estudio, la ausencia de diferencias significativas en los valores medios de $\delta^{18}O_{hueso}$ entre las etapas de desarrollo y entre sexos de los lobos marinos sudamericanos procedentes del norte de Patagonia, sugiere que los efectos de estos factores son mínimos. Por lo tanto, la variación regional de los valores $\delta^{18}O$ del agua de mar constituiría la fuente de variabilidad sobre las diferencias observadas entre los machos de Brasil, Patagonia y Tierra del Fuego. Sobre la base de esta información, los resultados aquí presentados sugieren que los machos adultos son bastante fieles a una región costera en particular, al menos durante varios años, pues las diferencias latitudinales detectadas en los valores de $\delta^{18}O$ en el hueso de los machos fueron coherentes con los patrones latitudinales de salinidad (Guerrero & Piola 1997) y con los valores regionales de $\delta^{18}O$ en el agua de mar (Bowen 2010).

Cabe resaltar que, en comparación con los machos de Brasil y Patagonia, la desviación estándar (SD) fue significativamente mayor en los machos de Tierra del Fuego, lo cual podría estar relacionado a un alto grado de variabilidad individual en el uso de los canales fueguinos (con baja salinidad) y las aguas más saladas del océano Atlántico (Guerrero & Piola, 1997).

La ausencia de diferencias en los valores medios de $\delta^{18}O_{hueso}$ entre hembras y machos adultos de la Patagonia sugiere que estos individuos comparten las mismas masas de agua durante todo el año. Sin embargo, esto no necesariamente implica que estén utilizando las mismas zonas de alimentación, ya que los valores de $\delta^{18}O_{aguademar}$ sobre la plataforma continental del océano atlántico sudoccidental varían con la latitud, pero no con la longitud (Bowen 2010). Por lo tanto, los animales que están utilizando zonas de alimentación a diferentes distancias de la costa, pero en la misma latitud, no difieren en sus valores de $\delta^{18}O_{hueso}$, lo cual explica por qué las hembras y los machos adultos de la Patagonia muestran valores de $\delta^{18}O_{hueso}$ similares, aunque las hembras se alimentan más cerca de la costa que los machos (Campagna et al. 2001). Lamentablemente esta conclusión no se puede extrapolar a Brasil y Tierra del Fuego, ya que en estas regiones solo se analizaron machos adultos, debido a la falta de cráneos de hembras en las colecciones.

En cambio, la disponibilidad de muestras de hueso de diferentes edades y sexo en las colecciones de Patagonia permitió evaluar las diferencias ontogénicas en la duración de la fase de dispersión de los lobos marinos sudamericanos. En general no se encontraron diferencias significativas en los valores medios de $\delta^{18}O_{hueso}$ entre los individuos maduros (adultos y seniles) y los jóvenes de ambos sexos, aunque estos últimos mostraron mayor variabilidad. Asimismo, los machos adolescentes también mostraron alta variabilidad en comparación con las hembras adolescentes.

La alta variabilidad detectada en los valores de $\delta^{18}O_{hueso}$ de los jóvenes revela la presencia en la misma región de individuos con diferentes historias alimentarias en los años previos a su muerte. Los mamíferos lactantes están enriquecidos con el isótopo pesado de nitrógeno en comparación con sus madres, fenómeno conocido como marca de lactancia. Una vez que se produce el destete, la intensidad de la marca de lactancia decae con el tiempo, para desaparecer totalmente tras varios meses (Newsome et al. 2010). Por lo tanto, es probable que la variabilidad de los individuos jóvenes analizados pueda ser debida a las diferencias de edad y en consecuencia en la intensidad de la marca de lactancia. No obstante, cabe destacar que aunque algunos estudios experimentales confirman la existencia de una señal de lactancia en los valores $\delta^{18}O_{hueso}$, la evidencia todavia es ambigua (Williams et al. 2005; Kirsanow & Tuross 2011).

Estudios anteriores han revelado que los machos adolescentes de los lobos marinos sudamericanos ya no muestran la señal de lactancia en la razón isotópica del nitrógeno (Drago et al. 2009a), por lo que la alta variabilidad detectada en la razón isotópica de oxígeno de estos individuos en comparación con los machos de edad más avanzada, debería tener otra explicación. En general, los resultados indican que los valores de $\delta^{18}O_{hueso}$ de los jóvenes de ambos sexos y de los machos adolescentes se superponen con el rango de los valores $\delta^{18}O_{hueso}$ de los machos adultos en toda el área de estudio (del sur de Brasil a Tierra del Fuego), lo que se sugiere que los individuos jóvenes de ambos sexos tienen una fase de dispersión previa a su asentamiento y que dicha área de dispersión abarca gran parte del rango de distribución de la especie.

Por otra parte, los marcadores genéticos sugieren filopatria por parte de las hembras de lobo marino sudamericano, pero no de los machos, los cuales se dispersarían más ampliamente y serían los responsables del flujo génico (Szapkievich et al. 1999; Freilich 2004; Túnez et al. 2007, 2010; Artico et al. 2010; Feijoo et al. 2011). La repentina disminución de la variabilidad de los valores de $\delta^{18}O_{hueso}$ en las hembras al alcanzar la edad adulta no puede ser explicada por la remodelación ósea (Schwarcz & Schoeninger 1991; Ambrose & Norr 1993), por lo que es probable que las hembras se asienten en los lugares donde nacieron. Por otro lado, la variabilidad de los valores de $\delta^{18}O_{hueso}$ de los machos disminuye lentamente a medida que crecen, de acuerdo con lo esperado en función de la tasa de renovación de la apatita, lo que sugiere que estos especímenes no necesariamente vuelven a sus lugares de nacimiento para asentarse.

En general, los resultados de este estudio señalan la existencia de una fase juvenil de dispersión en ambos sexos, aunque mucho más corta en las hembras que en los machos, lo cual es consistente con los datos disponibles sobre otras especies de pinnípedos (Burg et al. 1999; Hoffman et al. 2006; González-Suárez et al. 2009). Estos resultados coinciden con la información obtenida mediante marcadores genéticos, indicando un aislamiento de la población por distancia y un flujo de genes mediado por los machos, pero también sugieren que una vez que los machos han alcanzado la edad reproductiva se asientan lejos de sus lugares de nacimiento y, por lo que serian los principales responsables del flujo de genes. Además, estos resultados demuestran que una vez asentados, los animales adultos de ambos sexos permanecen en la misma región. Por lo tanto, y de acuerdo con la variabilidad regional existentes en la línea de base isotópica para C y N (Saporiti et al. 2015), para los estudios posteriores realizados en el seno de esta tesis se decidió tratar por separado las muestras procedentes de la pluma del Río de la Plata (de Buenos Aires al sur de Brasil), el norte de Patagonia (Chubut y norte de Santa Cruz) y el sur de Patagonia (sur de Santa Cruz y Tierra del Fuego).

Cambios en las razones isotópicas de tejidos inertes

En el segundo capítulo de esta tesis se pretendía determinar hasta qué punto las variaciones isotópicas en los tejidos inertes corresponden únicamente a cambios en la dieta. Los análisis previos de la señal isotópica en vibrisas de fócidos y otáridos mantenidos en cautividad habían revelado una baja variación en los valores de $\delta^{13}C$ y $\delta^{15}N$ en ejemplares mantenidos a dieta constante (Hobson et al. 1996; Hirons et al. 2001b), y en consecuencia las fluctuaciones cíclicas observadas en otáridos silvestres se habían interpretado simplemente como evidencia de cambios periódicos de dieta o debido a cambios de las zonas de alimentación (Hirons et al. 2001b; Cherel et al. 2009; Kernálegen et al. 2012, 2015, Vales et al. 2015; Baylis et al. 2015). Sin embargo, estudios realizados en otros mamíferos habían observado cambios cíclicos en las razones isotópicas asociados a procesos de ayuno (Beltrán et al. 2015), lactancia (Jenkins et al. 2001) y gestación (Fuller et al. 2004), por lo tanto, cabía esperar que estos procesos pudieran contribuir de forma importante a la variabilidad de las razones isotópicas.

Los resultados aquí presentados corroboran esta hipótesis, ya que se observaron importantes oscilaciones periódicas en los valores de $\delta^{13}C$ y $\delta^{15}N$ a lo largo de las vibrisas de los lobos marinos sudamericanos adultos cautivos alimentados con una dieta constante. Estas oscilaciones se reflejaron en los machos, con una caída de los valores de $\delta^{13}C$ y $\delta^{15}N$ durante la época de celo, mientras que en las hembras los valores de $\delta^{13}C$ aumentaron durante el período central de la gestación, y los valores de $\delta^{15}N$ aumentaron durante la lactancia.

Los machos sexualmente activos de los lobos marinos sudamericanos pueden pasar hasta 40 días sin alimentarse durante la temporada reproductiva en el verano austral (Campagna 1985) y el macho adulto estudiado en cautividad en esta tesis también redujo significativamente la ingesta de alimentos durante los meses del verano boreal.

La existencia de variaciones en la señal isotópica como resultado de los procesos fisiológicos que regulan el ayuno ha sido evaluada en numerosos trabajos previos sobre diferentes especies de vertebrados homeotermos, con resultados dispares (Hobson et al. 1993; Hobson et al. 1993; Lockyer, 1987; Gómez-Campos et al 2011; Aguilar et al. 2014). Uno de los primeros estudios sobre el tema sugirió que, en las aves, el ayuno no causaba ningún cambio en los valores de $\delta^{13}C$, pero sí un importante enriquecimiento en ^{15}N debido al catabolismo de los aminoácidos endógenos, a fin de satisfacer las demandas energéticas durante los períodos de estrés nutricional, gracias a la degradación protéica, y a la excreción preferencial subsiguiente de ^{14}N (Hobson et al. 1993). Sin embargo, en algunos mamíferos marinos, la gruesa capa de grasa subcutánea permitiría la conservación de las proteínas, incluso durante periodos de ayuno prolongados, y en consecuencia no se generaria ningún cambio en las razones isotópicas (Gómez-Campos et al. 2011) o incluso una disminución en los valores de $\delta^{13}C$ y $\delta^{15}N$ durante los periodos de estrés nutricional (Polischuk et al. 2001; Aguilar et al. 2104; Matthews & Ferguson 2015; Vales et al. 2105).

Cualquiera que sea la explicación fisiológica real para estos cambios, en el presente estudio se identificaron marcadas oscilaciones en los valores de $\delta^{13}C$ y $\delta^{15}N$ a lo largo de las vibrisas del macho mantenido en cautividad, siendo los valores más bajos los registrados durante el periodo de ayuno, lo cual apoya fuertemente la hipótesis de que el ayuno genera importantes caídas en los valores de $\delta^{13}C$ y $\delta^{15}N$ de los mamíferos marinos, quizás en relación con la necesidad de evitar el estrés hídrico (Aguilar et al. 2014). Aunque sólo se estudió un macho en cautividad, los valores de $\delta^{13}C$ y $\delta^{15}N$ de los machos adultos salvajes también fluctuaron periódicamente y las mayores caídas en los valores de $\delta^{13}C$ y $\delta^{15}N$ resultaron estar asociadas al prolongado período de ayuno durante la temporada de apareamiento.

Las hembras lactantes de otáridos cuentan con escasas reservas de grasa en sus cuerpos en comparación con las hembras de fócidos, y por consiguiente alternan la lactancia con periodos de alimentación en el mar para sintetizar leche rica en lípidos y asegurar la sobrevivencia de las crías (Berta & Sumich 1999). En otaridos, y otros mamíferos que se comportan como *capital breeders*, la proteína de la leche se empobrece tanto en ^{13}C como en ^{15}N en comparación con la sangre en las hembras lactantes (Jenkins et al. 2001; Cherel et al. 2015). Por ello, se debería observar un enriquecimiento en ^{13}C y ^{15}N de los tejidos de las hembras a medida que avanza la lactancia. Dicho enriquecimiento en ^{13}C sería particularmente marcado en hembras lactantes de otáridos, ya que producen leche con alto contenido de lípidos (Berta & Sumich 1999) y empobrecida en ^{13}C en comparación con los otros tejidos (Newsome et al. 2006). No obstante, la situación es diferente en hembras reproductoras que producen descendencia utilizando las reservas de energía acumuladas anteriormente y ayunan durante la lactancia (Polischuck et al. 2001; Ducatez et al. 2008; Habran et al. 2010; Borrell et al. 2016), debido a que probablemente el ayuno y la lactogénesis tiene efectos opuestos en las razones isotópicas de las hembras.

Los resultados del análisis de la señal isotópica de la hembra en cautividad coinciden con las predicciones mencionadas anteriormente para las hembras de los otáridos. En general, este estudio revela un leve incremento en los valores de $\delta^{13}C$ y $\delta^{15}N$ desde el parto hasta el destete durante los dos ciclos de lactancia, pero con un marcado incremento en los valores de $\delta^{13}C$ durante la gestación. En esta situación, discriminar entre las fuentes de variabilidad, ya sea fisiológicas o dietarias, puede ser difícil en otáridos silvestres. Los diez adultos silvestres aquí estudiados mostraron oscilaciones periódicas de $\delta^{13}C$ y $\delta^{15}N$ a lo largo de las vibrisas, así como también las hembras estudiadas por Baylis et al. (2015) en las Islas Malvinas / Falkland. Por otra parte, en los machos el ayuno durante el período de celo probablemente explique la mayor parte de la variabilidad en los valores de $\delta^{15}N$, mientras que la variabilidad en los valores de $\delta^{13}C$ podría estar relacionado con los cambios de dieta.

El impacto de los cambios en la dieta fue probablemente mayor en las hembras, no sólo debido a que la variabilidad de $\delta^{13}C$ y $\delta^{15}N$ fue mayor a lo que se esperaría del ciclo anual reproductivo, sino que también debido a que los valores de $\delta^{13}C$ y $\delta^{15}N$ oscilaron simultáneamente, lo cual no sucedió en cautividad.

Drago et al (2010a) sugirieron que las hembras de lobo marino sudamericano del norte de la Patagonia consumen preferentemente presas pelágicas u oceánicas antes del parto, para cambiar luego a presas demersales o costeras. En el norte de la Patagonia, las presas pelágicas se encuentran empobrecidas en ^{13}C y ^{15}N en comparación con las presas bentónicas (Drago et al. 2010a) y, por lo tanto, se espera encontrar que los valores de δ^{13}C y δ^{15}N en las vibrisas de las hembras muestren más los cambios de dieta que los cambios fisiológicos relacionados con el ciclo reproductivo anual. La existencia de una oscilación simultánea en hembras silvestres, pero no en las hembras en cautividad, apoya esta hipótesis. Además, se han observado oscilaciones similares en hembras que explotan zonas de alimentación costeras de las islas Malvinas / Falkland, mientras que las oscilaciones observadas en las hembras que explotan zonas más oceánicas son de menor amplitud (Baylis et al. 2015). Esto también es coherente con la existencia de los movimientos oceánicos/costeros durante el ciclo anual de los individuos con hábitos alimenticios costeros.

En conclusión, tanto los cambios en la dieta como los procesos fisiológicos relacionados con el ciclo reproductivo pueden influir en las razones isotópicas de las vibrisas de otáridos y por ende en las de otros tejidos metabólicamente inertes. Ahora bien, la ausencia de diferencias en la señal isotópica promedio de las vibrisas de machos y hembras cautivos sugieren que dichas variaciones fisiológicas se equilibran a lo largo del año y que no afectan a los valores medios observados en tejidos como el hueso, que integra la dieta durante varios años (Riofrío-Lazo and Aurioles-Gamboa 2013).

Una vez aclaradas estas cuestiones metodológicas, se pudo abordar en el tercer y cuarto capítulo la cuestión principal planteada en esa tesis.

Efectos de la explotación humana sobre la dieta de los lobos marinos sudamericanos

El análisis efectuado en el tercer capítulo constituye una nueva aproximación a la reconstrucción histórica de la dieta del lobo marino sudamericano, realizada a partir de los datos zooarqueológicos disponibles para la región. Gracias al análisis de las valvas de moluscos subfósiles pertenecientes a diferentes momentos del Holoceno medio y tardío, fue posible detectar importantes variaciones en la línea de base isotópica en cada región y periodo. Por ello, fue necesario corregir la señal isotópica de los huesos modernos y antiguos de los lobos marinos sudamericanos de diferentes períodos antes de poder compararlos entre sí.

Los resultados indican que desde el Holoceno tardío hasta hoy, existió un aumento del δ^{15}N del hueso tanto en el norte como en el sur de la Patagonia, pero de forma más marcada en el norte. En cambio, los valores de δ^{13}C aumentaron de manera significativa sólo en el norte. Por otra parte, los resultados también mostraron que las presas demersales de la Patagonia norte-centro estuvieron más enriquecidas tanto en ^{13}C y como en ^{15}N, que los peces pelágicos de la misma región. En consecuencia, el aumento simultáneo de δ^{13}C y δ^{15}N en los huesos de los lobos marinos sudamericanos de esa región sugiere un aumento reciente en el consumo de presas demersales.

En el sur de Patagonia, la señal isotópica de nitrógeno en el hueso de los lobos marinos sudamericanos no cambió a lo largo de todo el Holoceno medio y tardío, pero aumentó durante el siglo XX, lo cual sugiere un incremento del nivel trófico de las presas consumidas con respeto a las épocas anteriores. La mayoría de las presas pelágicas y demersales del sur de la Patagonia mostraron valores similares de δ^{13}C y δ^{15}N, por lo que es poco probable que el cambio en el δ^{15}N en el hueso de estos individuos se deba a un cambio en el conjunto de las presas y podría estar más relacionado con un incremento en el consumo de especies de nivel trófico más elevado, como la merluza (*Merluccius hubbsi*).

Por otra parte, la señal isotópica correspondiente a las décadas de 1970 y 1980, indica un consumo de grandes presas demersales cuando la población de lobo común se hallaba en su mínimo histórico, precisamente las presas teóricamente más rentables en términos energéticos. En cambio, la dieta correspondiente a las décadas de 1990 y 2000 se ha desplazado hacia el incremento en el consumo de presas de menor valor energético, como la raneya (*Raneya brasiliensis*) o los juveniles de merluza (*M. hubbsi*), lo que puede explicarse por el incremento de la población de lobo común y la consecuente disminución de la abundancia *per capita* de presas demersales de elevado valor.

En general los resultados del presente estudio muestran que los lobos marinos sudamericanos se alimentan hoy a un nivel trófico más elevado que durante el Holoceno tardío. Los datos también revelan al siglo XX como el período en que se produjo la mayor parte de dicho cambio del nivel trófico, coincidiendo con la eliminación masiva de individuos por la caza lo largo de la costa de Argentina (Dans et al. 2004; Schiavini et al. 2004; Grandi et al. 2012). En cambio, la estabilidad de la dieta detectada durante el periodo de explotación indígena apoya la hipótesis de que la caza aborigen no habría provocado cambios en la dieta de los lobos marinos sudamericanos en Argentina.

Cambios históricos en la dieta de los lobos marinos sudamericanos

Una vez comprendido cómo ha variado la posición trófica del lobo marino sudamericano a lo largo del Holoceno medio y tardío, se abordó el análisis de la dieta durante tres décadas en el sur de Brasil con el fin de determinar cómo había afectado el desarrollo de la industria pesquera del Río de la Plata y zonas adyacentes y así complementar los datos disponibles sobre el norte del Patagonia (Drago et al. 2009a). A pesar que esta región cuenta con una importante pesquería demersal, los desembarques de biomasa, la captura por unidad de esfuerzo y el nivel trófico medio de los desembarques disminuyeron a mediados de 1990 en algunas zonas y actualmente son muchas las poblaciones de peces clasificadas como completamente explotadas o sobreexplotadas (Haimovici 1998; Vasconcellos & Gasalla 2001; Jaureguizar & Milessi 2008; Milessi & Jaureguizar 2013). Como respuesta a lo anterior, el delfín franciscana (*Pontoporia blainvillei*) disminuyó el consumo de peces demersales (Pinedo 1994; Secchi et al. 2003; Crespo & Hall 2002) y la contribución general de los peces demersales en la dieta de las aves marinas disminuyó a lo largo de los últimos 30 años (Bugoni 2008).

Sin embargo, los lobos marinos sudamericanos provenientes del sur de Brasil muestran un comportamiento opuesto a lo descrito anteriormente para otros depredadores apicales de la región, ya que durante un periodo de 30 años no se observaron grandes cambios en la dieta de machos jóvenes ni de adultos.

La señal isotópica observada en la mayor parte de los machos del sur de Brasil aquí analizados indica un nicho isotópico conformado por una mezcla de presas demersales y pelágicas, aunque existe un alto nivel de variabilidad individual durante todo el periodo de estudio. Se proponen dos posibles causas para esta variabilidad. En primer lugar, los individuos que se reproducen en Uruguay se alimentan en una amplia zona que abarca desde el sur de Brasil hasta el norte de Argentina (Rodríguez et al. 2013), y las presas de esas regiones difieren en sus señales isotópicas (Abreu et al. 2006; Bugoni et al. 2010; Botto et al. 2011; Franco-Trecu et al. 2013a; este estudio). Además, no es posible descartar la existencia de diferencias individuales en la estrategia de alimentación, ya que las razones isotópicas de algunos machos adultos están más cerca a la de los peces pelágicos de tamaño medio que a la de los peces demersales. En cualquier caso, el análisis de contenidos estomacales (Oliveira et al. 2008; Machado 2013) e isótopos estables (este estudio), identifican a los peces demersales como el alimento básico de los especímenes procedentes del Sur de Brasil, a pesar de la alta abundancia de presas pelágicas en el área.

Existen al menos dos explicaciones no excluyentes entre sí para esto. Primero, una selección basada en el tamaño de las presas, ya que las presas bentónicas generalmente son más grandes que las presas pelágicas. Segundo, la preferencia por presas bentónicas podría ser explicada por el comportamiento más sedentario de los lobos (Womble & Sigler 2006) en contraste con el permanente movimiento de las presas pelágicas (Gende & Sigler 2006). La primera hipótesis se ve apoyada por el gran tamaño de las presas demersales consumidas por el lobo marino sudamericano en comparación con el de las presas pelágicas (Szteren et al. 2004; Riet-Sapriza et al. 2013), que compensaría su menor densidad energética (Drago et al. 2009a). En este sentido, cabe señalar que los peces demersales también fueron claves en la dieta de los machos jóvenes y adolescentes, aunque los peces de tamaño pequeño y medio representaron el grueso de su dieta. Los cambios ontogénicos en la dieta de pinnípedos están a menudo relacionados con el crecimiento somático y la capacidad de buceo (Gentry et al. 1986; Horning & Trillmich 1997; Costa et al. 2004). Esta relación se observa también en el lobo marino sudamericano, ya que esta especie incrementa la profundidad de buceo a medida que crece y como resultado de esto, incrementa también el consumo de presas demersales (Drago et al. 2009b). Este patrón fue también observado en el presente estudio, pero no fue detectado en los análisis de excrementos de Uruguay (Naya et al. 2000; Szteren et al. 2004), según los cuales los pequeños peces pelágicos representarían el grueso de la dieta de la especie en Uruguay. Esta discrepancia podría estar señalando que los resultados de los análisis de excrementos estarían representando en gran medida, la dieta de individuos jóvenes y adolescentes, generalmente presentes en la periferia de las colonias de cría y por lo tanto sobre representados en las muestras de excrementos recogidas de forma no intrusiva.

La estabilidad de la dieta durante las tres últimas décadas observada en el sur de Brasil, Uruguay y norte de Argentina, donde la población de lobos marinos sudamericanos es pequeña y en retroceso, se contrapone a los cambios de dieta observados en norte y centro de la Patagonia argentina durante el siglo XX, donde los lobos han incrementado el consumo de presas pelágicas desde 1970 (Koen-Alonso et al. 2000; Drago et al. 2009a; Romero et al. 2011) en paralelo al desarrollo de las pesquerías de arrastre de fondo, y al incremento de su población, como resultado de la protección legal (Drago et al. 2009a). Esto sugiere que el drástico cambio de dieta observado en los ejemplares del norte de la Patagonia podría deberse a la disminución de la disponibilidad per cápita de presas demersales, por lo que es probable que la dinámica poblacional sea un factor clave en la alimentación de esta especie (Drago et al., 2009a).

Una de las ventajas de los análisis de isótopos estables es que son más efectivos para detectar cambios de dieta a largo plazo, en comparación con los análisis de excrementos y contenidos estomacales, ya que estos últimos proporcionan sólo una "instantánea" de la dieta de cada individuo justo antes de cada muestreo (Iverson et al. 2004). Por otra parte, para evitar errores en la interpretación de los estudios de series temporales, es necesario disponer de datos sobre las posibles variaciones en la señal isotópica de base (Casey & Post 2011). En este estudio, los valores fueron corregidos tomando en cuenta el efecto Suess (Francey et al. 1999; Indermühle et al. 1999), pero no se disponía de muestras de referencia históricas de peces y vertebrados que permitieran controlar otras fuentes de variación. En cualquier caso, la evidencia actual revela que no existieron importantes cambios en la productividad primaria del sur de Brasil desde 1998 a 2006 (Heileman & Gasalla 2008), y en consecuencia, no se esperarían cambios importantes en los valores de $\delta^{15}N$ de los productores primarios, ya que ambos parámetros están fuertemente correlacionados a lo largo del Atlántico sudoccidental (Saporiti et al. 2014).

Otro factor limitante que podría estar influenciando la interpretación de los resultados en este estudio, es la utilización de dos tejidos que integran la información sobre diferentes periodos de tiempo. Por un lado, los huesos de pinnípedos integran información alrededor de 5 años de la dieta, mientras que la dentina de los caninos integran la dieta de toda la vida (Riofrío-Lazo & Aurioles-Gamboa 2013). La diferencia se debe a que los huesos son metabólicamente activos y están sometidos a una tasa de renovación constante, mientras que la dentina es metabólicamente inerte y nuevas capas se asientan dentro de la cavidad pulpar de los dientes caninos a lo largo de la vida del individuo (Riofrío-Lazo & Aurioles-Gambioa 2013). La señal de lactancia permanece indefinidamente en la dentina una vez que se ha formado durante el primer año de vida, pero se desvanece en el hueso después del primer o segundo año debido a la tasa de renovación del tejido (Drago et al. 2009b; Newsome et al. 2010a). De acuerdo con esto, las reconstrucciones de la dieta usando dentina y hueso en individuos mayores a 2 años pueden llevar a diferentes conclusiones en relación a nivel trófico. Sin embargo, el impacto de la señal de lactancia sobre las razones isotópicas de la dentina disminuyen una vez que las nuevas capas se van acumulando (<1/9 de la dentina), por lo que se descarta influencia alguna de la señal isotópica en los individuos adultos.

La pesca es actualmente el principal factor de cambio de los ecosistemas costeros en el Atlántico sudoccidental (Koen-Alonso & Yodzis, 2005) y como consecuencia del desarrollo pesquero, la capacidad de carga de los ecosistemas para los lobos marinos sudamericanos ha variado (Drago et al. 2010a). La presente tesis doctoral ha demostrado que es posible reconstruir los cambios históricos en la dieta de los depredadores marinos mediante el análisis de isótopos estables en hueso y que los lobos marinos sudamericanos del Atlántico sudoccidental se alimentan en un nivel trófico más elevado del que ocuparon originalmente. Por otra parte, la mayor parte de este cambio estuvo relacionado con la reducción de sus poblaciones fruto de la la explotación humana durante el siglo XX, habiéndose producido sólo modificaciones menores durante el propio siglo XX. Por lo tanto, se concluye que a pesar de los avances en la protección jurídica de la especie, estamos aún lejos de restaurar su función ecológica.

CONCLUSIONES

“ El error consistió en creer que la tierra era nuestra, cuando la
verdad de las cosas es que nosotros somos de la tierra”
Nicanor Parra

•El análisis de los isótopos estables de oxígeno en la apatita del hueso de los lobos marinos sudamericanos mostró ser una metodología útil como trazadora de hábitats en mamíferos marinos, ya que permitió determinar que los movimientos de dispersión de los machos adultos se encuentran restringidos entre las regiones de Brasil, Patagonia y Tierra del Fuego en Argentina.

•Los valores de $\delta^{18}O$ en huesos de machos y hembras adultos de Patagonia sugieren el uso de las mismas masas de agua como zonas de alimentación por parte de ambos sexos.

•Los individuos jóvenes de ambos sexos mostraron una variabilidad mayor en los valores de $\delta^{18}O$ del hueso en comparación con los adultos, lo que sugiere la existencia de un fase juvenil de dispersión en ambos sexos, aunque mucho más corta en las hembras que en los machos.

•Las razones isotópicas de C y N en las vibrisas de lobos marinos sudamericanos mantenidos en cautividad fluctúan de forma periódica, por lo que la dieta no constituye la única fuente de variación en los ejemplares salvajes.

•Los valores de $\delta^{13}C$ y $\delta^{15}N$ muestran marcadas oscilaciones a lo largo de las vibrisas de los machos mantenidos en cautividad y los silvestres, las cuales coincidieron con los periodos de ayuno durante la temporada de apareamiento.

•En las hembras en cautiverio se observó un leve incremento en los valores de $\delta^{13}C$ y $\delta^{15}N$ desde el parto hasta el destete durante los dos ciclos de lactancia, pero con un marcado incremento en los valores de $\delta^{13}C$ durante la gestación. En cambio, en las hembras salvajes se observó un descenso simultáneo de los valores del $\delta^{13}C$ y $\delta^{15}N$ durante la lactancia, indicativo de un cambio de dieta.

•El análisis de los isótopos estables de carbono y nitrógeno en muestras zooarqueológicas y modernas permitió detectar un cambio en el nicho trófico de los lobos marinos sudamericanos desde el Holoceno tardío hasta el presente. Tanto en Patagonia norte-centro con el Patagonia sur, las poblaciones de lobos marinos sudamericanos mostraron una estabilidad en la dieta durante varios milenios de explotación aborigen. Sin embargo, durante el siglo XX se observó un marcado aumento del nivel trófico, el cual podría estar relacionado a una disminución del tamaño poblacional del lobo común sudamericano, como resultado de la explotación comercial, y una reducción de la competencia intraespecífica.

•No se observaron cambios significativos durante las últimas tres décadas en las razones isotópicas del hueso de machos jóvenes y adultos de lobo marino sudamericano procedentes del sur de Brasil a pesar de un dramático incremento en el desarrollo de la pesquería demersal. Esto sugiere que la población regional se encuentra muy por debajo de la capacidad de carga.

•Los análisis retrospectivos de las razones isotópicos permiten inferir cambios históricos en la dieta de los depredadores oportunistas, lo que resulta clave a la hora de comparar su ecología en ecosistemas prístinos y modificados antropogénicamente.

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ciencia , y sin ciencia no hay desarrollo”
Lisette Zenteno

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