UNIVERSITAT DE BARCELONA

Estructura y migración del rorcual común del Atlántico nororiental establecido mediante trazadores químicos

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Estructura y migración del rorcual común del Atlántico nororiental establecido mediante trazadores químicos

Memoria presentada por

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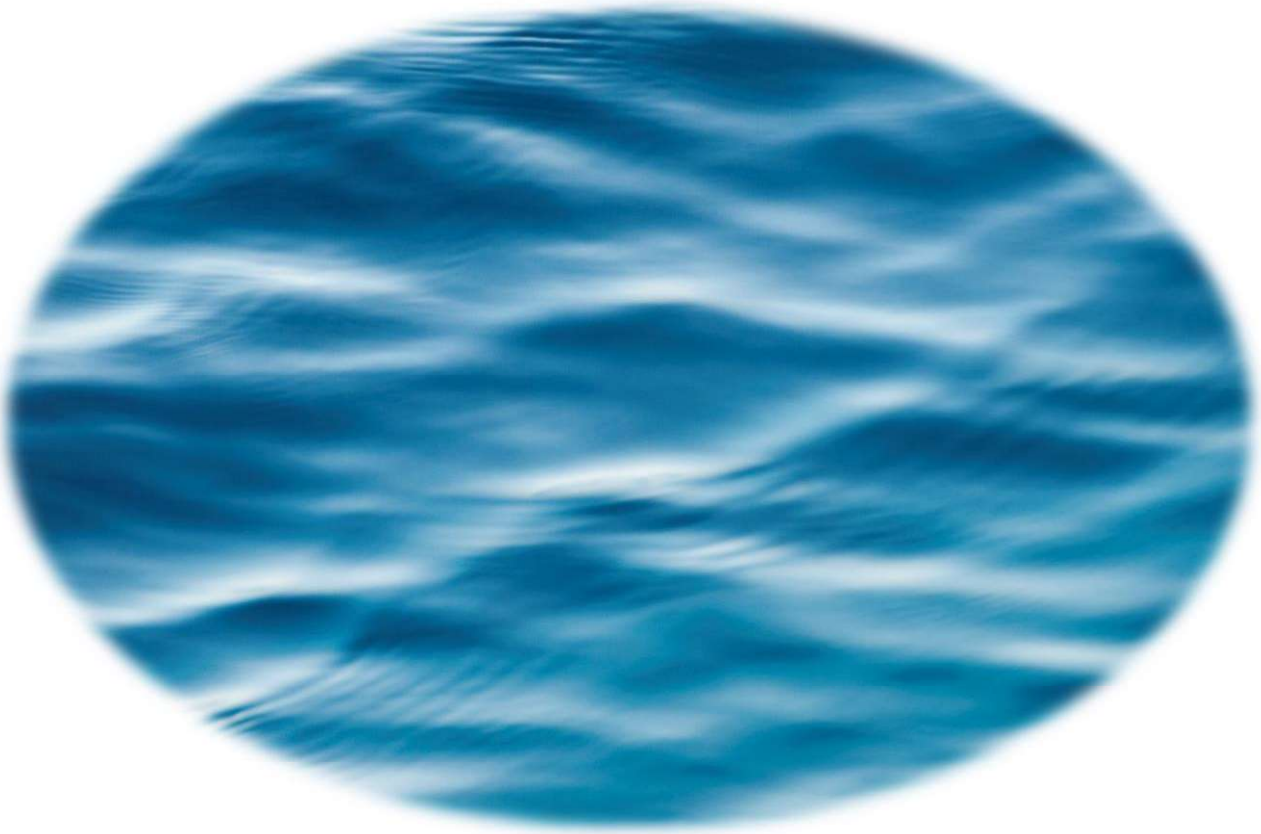
Abstract

Fin whales (*Balaenoptera physalus*) is one of the most abundant mysticete species in the North Atlantic, and it is considered a species of high environmental importance by the Spanish legislation. However, large knowledge gaps exist in the biology of this species, especially in winter. The main goal of this thesis is to improve our understanding of the migrations of the fin whale analyzing chemical tracers in three different tissues. In the first chapter, alkenones were analyzed in blubber and the stomach content of the fin whales from Iceland and the NW of Spain. The alkenones are a group of organic molecules produced by some haptophyte species, and may be used to infer the water temperature where they were synthesized. The results show that these molecules can be transferred through the trophic web and detected both in the stomach content and the blubber of the whales. While the temperature estimated in the stomach content reflected the environmental SST 10 days before the sampling date, the alkenones in the blubber reflected the environmental temperature where the fin whales had roamed during winter. In the second chapter, the stable isotopes of amino acids were analyzed in baleen plate samples. The results showed that the trophic level of the individuals were higher and more variable during winter, which could indicate that they were including fish in their diet during the winter season. Furthermore, the baseline isotopic values suggested that the fin whales spent the winter season in zones of deep water emergence, which can support a higher primary production than the oligotrophic ocean. In the third chapter, the temporal consistency of the stable isotopes was measured in the earplugs of fin whales. The results showed that the fin whales are individual specialists, this is, they occupy a small portion of the population isotopic niche. Overall, the fin whale stocks studied in this thesis are more generalist during winter than during summer, however, the individuals tend to migrate and feed in specific zones every year, which are characterized by a high primary production.

Resumen

El rorcual común (*Balaenoptera physalus*) es una de les especies de misticeto más abundante en el Atlántico Norte y es considerada como una especie de alta importancia ambiental por la legislación española. Sin embargo, aún quedan grandes lagunas de conocimiento en la biología de esta especie, especialmente durante el invierno. El objetivo global de esta tesis es la mejor comprensión de la estructura poblacional y las migraciones del rorcual común mediante el análisis de marcadores químicos en tres tejidos diferentes. En el primer capítulo, se analizaron alquenonas en la grasa hipodérmica y en el contenido estomacal de rorcuales de Islandia y del noroeste español. Las alquenonas son moléculas orgánicas producidas por organismos haptófitos que pueden ser usadas para estimar la temperatura del agua donde han sido sintetizadas. Los resultados muestran que estas moléculas pueden ser transferidas a lo largo de la red trófica y ser detectadas tanto en el contenido estomacal de los rorcuales como en la grasa hipodérmica. Mientras la temperatura estimada en el contenido estomacal refleja la temperatura ambiental de los 10 días antes del muestreo, las alquenonas de la grasa hipodérmica parecen reflejar la temperatura del hábitat de los rorcuales durante el invierno. Los rorcuales de las dos áreas estudiadas mostraron una composición de alquenonas bien diferenciada, lo que indica que se trata de poblaciones segregadas. En el segundo capítulo se analizaron los isótopos estables en aminoácidos muestreados en las barbas de ballena. Se observó que el nivel trófico de los rorcuales se vuelve más alto y variable durante el invierno, lo que podría indicar que esta especie complementa su dieta con pescado durante esta época. Además, los valores isotópicos ambientales del invierno sugieren que los rorcuales pasan la época invernal en zonas de emergencia de agua profunda, las cuales pueden sostener una producción primaria mayor que el resto del océano oligotrófico. En el tercer capítulo se midió la consistencia temporal de los isótopos estables en los conos auditivos de los rorcuales. Estos mostraron que los rorcuales son especialistas individuales, es decir los individuos ocupan una porción del nicho poblacional disponible. En conjunto, los stocks estudiados de rorcual común del Atlántico Norte parecen ser más generalistas durante el invierno, pero los individuos parecen migrar i alimentarse en zonas concretas cada año, caracterizadas por una alta producción primaria.

Índice



Introducción General

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Como su nombre común indica, el rorcual común (*Balaenoptera physalus*) es el rorcual más abundante en las costas españolas y uno de los más abundantes en el Atlántico norte. Durante el siglo XX, fue una de las especies más explotadas por las compañías balleneras hasta que la moratoria de la Comisión Ballenera Internacional (IWC) detuvo la caza comercial de esta especie casi por completo. Desde entonces, la mayoría de poblaciones de rorcual común se han recuperado y la IUCN considera esta especie como *vulnerable* (Cooke 2018). En España, esta especie está considerada como una especie indicadora del "buen estado ambiental" del medio marino por la Directiva Marco sobre la Estrategia Marina. Sin embargo, a pesar de la abundancia de esta especie en las aguas del Atlántico Norte, de su explotación en el pasado y de su importancia como especie indicadora en la actualidad, todavía quedan grandes incógnitas sobre su biología. En concreto, se puede destacar el desconocimiento en la biología de esta especie durante el invierno. La presente tesis doctoral tiene por objetivo usar marcadores químicos para ampliar el conocimiento actual sobre el rorcual común, poniendo especial énfasis en las estrategias de migración y su comportamiento durante la estación invernal.

Rorcual común

El rorcual común es uno de los misticetos más abundantes del Atlántico norte. Su población actual se estima en más de 79.000 individuos (Cooke 2018), a los que habría que añadir unos 3.600 más en el mar Mediterráneo (Forcada et al. 1996). De hecho, algunos autores consideran que en esta región la población se había recuperado completamente ya en el año 2000 (Víkingsson et al. 2015). La división de estos individuos en poblaciones es un tema aún por resolver. La IWC reconoció 7 stocks de gestión del rorcual común en el Atlántico Norte basándose en evidencias genéticas i no-genéticas (International Whaling Comission 2009) (Figura 1). Esta división se basó principalmente en una separación de las zonas de alimentación visitadas por esta especie, que es donde se realizan la gran mayoría de estudios. A pesar de esta división, es muy posible que individuos de diferentes stocks se reproduzcan entre ellos. De hecho, la misma reunión de la IWC que definió los stocks sugirió que estos 7 stocks posiblemente se combinarían durante el invierno para formar 4 stocks reproductivos (International Whaling Comission 2009). Es decir, los dos stocks del Atlántico Norte occidental (Este de Canadá y oeste de Groenlandia) compartirían una zona de reproducción común, al igual que los tres stocks centrales (este de Groenlandia, Oeste de Islandia y este de Islandia + Islas Feroe),

mientras que los stocks español y noruego tendrían cada uno una zona de reproducción más o menos independiente (Árnason 1995). El grado de entrecruzamientos entre los 4 stocks reproductivos es desconocido y, si se produjera, se podría dar tanto entre stocks vecinos como entre todos los stocks del Atlántico Norte.

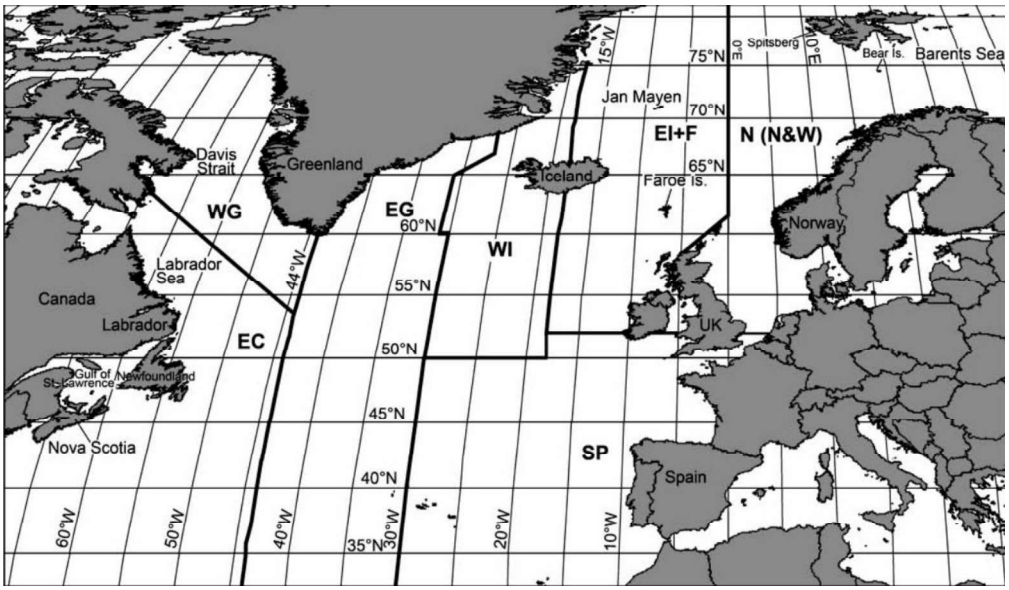


Figura 1 Stocks de rorcual común del Atlántico Norte definidos por la Comisión Ballenera Internacional; EC: este de Canadá, WG: oeste de Groenlandia, EG: este de Groenlandia, WI: oeste de Islandia, EI+F: este de Islandia + Islas Feroe, N: Noruega, SP: España. Figura adaptada de IWC (2009).

El ciclo reproductor del rorcual común es anual para los machos pero bienal para las hembras. Durante el verano, tanto machos como hembras se agrupan en las zonas de veraneo, donde se alimentan, y, durante el invierno, la mayoría de los individuos migran hacia latitudes más bajas donde se produce la cópula (Baines et al. 2017; Aguilar and García-Vernet 2018). La gestación dura cerca de 11 meses y las crías nacen en el siguiente invierno, después de que la madre haya ido y vuelto a las zonas de alimentación durante el verano. Al nacer, madre y cría migran de las zonas de reproducción a las zonas de alimentación. Tras una lactancia de 7 meses, el destete se produce durante los meses de verano y la cría empieza a alimentarse por su cuenta (Mizroch et al. 1984). Tras el destete, la madre tiene un período de descanso de 6 meses hasta la siguiente época reproductiva (Aguilar and García-Vernet 2018). Las crías de rorcual común tardan entre 6 y 8 años en alcanzar madurez sexual (Aguilar et al. 1988) y entre 9 y 13 años en alcanzar su máximo tamaño corporal (Aguilar and Lockyer 1987).

La zona de invernada de los rorcuales comunes sigue siendo, en gran parte, un misterio. En las especies de misticetos mejor estudiadas, la ballena jorobada (*Megaptera novaeangliae*) y los balénidos, estas migraciones suelen ser estrictas y las zonas de veraneo y de invernada son conocidas y están bien acotadas (Clapham 2017; Kenney 2017). Los individuos, especialmente las hembras, tienen tendencia a migrar cada año de la misma zona de invernada a la misma zona de veraneo (Baker et al. 2013; Horton et al. 2017), ambas cercanas a la costa, lo que facilita su estudio. En muchos

balenópteros, sin embargo, parece que este modelo migratorio no se ajusta completamente (Geijer et al. 2016). Si bien algunas especies sí que se agrupan durante el verano en zonas de alta producción primaria para alimentarse, durante el invierno parece que los individuos se dispersan, con lo cual su presencia se hace más difícil de detectar (Nieukirk et al. 2012; Lesage et al. 2017; Lydersen et al. 2020). Una consecuencia de ello es que, a excepción de la pesquería que tuvo lugar en los años 1920s en el estrecho de Gibraltar, todas las operaciones balleneras realizadas han tenido lugar en las zonas de alimentación en verano. Muchos estudios han intentado resolver esta incógnita con diferentes metodologías. El despliegue de marcas satelitales ha sido un método muy utilizado (Watkins et al. 1996; Heide-Jørgensen et al. 2003; Silva et al. 2013; Víkingsson and Heide-Jørgensen 2015; Lesage et al. 2017; Lydersen et al. 2020), pero poco efectivo dado que estas marcas suelen desprenderse a las pocas semanas o meses. Como los estudios citados anteriormente muestran, se deben marcar una cantidad elevada de animales para que alguna de las marcas se mantenga durante todo el período de la migración. También se han utilizado los métodos acústicos (Charif and Clark 2009; Nieukirk et al. 2012; Romagosa et al. 2020), pero estos suelen tener una precisión muy baja y no descarta la presencia de animales que no estén realizando sonidos (Croll et al. 2002; Delarue et al. 2009).

Los estudios de migración realizados hasta la fecha en el rorcual común muestran que sus migraciones invernales son muy variables. En los mares casi cerrados, como el Mediterráneo o el Golfo de California, existen poblaciones residentes que no realizan migraciones latitudinales, o éstas son mínimas (Panigada et al. 2017; Jiménez López et al. 2019). En el caso del mar Mediterráneo, algunos individuos salen al Océano Atlántico durante el invierno (Gauffier et al. 2018; Pereira et al. 2020), pero la mayoría se queda dentro y forman pequeños grupos de alimentación (Canese et al. 2006). Algunas poblaciones oceánicas también muestran patrones migratorios peculiares, como la población que habitaba en el Golfo de Cádiz. Esta población fue intensamente explotada a principios del siglo XX (Sanpera and Aguilar 1992; Clapham et al. 2008) y los registros de las factorías balleneras muestran una falta total de estacionalidad en sus capturas (Clapham et al. 2008). Es decir, se capturaban el mismo número de individuos a lo largo de todo el año. Esto parece indicar que esta población no migraba y que podía pasar todo el año en la misma zona. Por desgracia, la población fue completamente explotada y no se ha recuperado a pesar del paso esporádico de rorcuales comunes por la zona (Clapham et al. 2008; Pereira et al. 2020). En el otro extremo encontramos los rorcuales comunes del hemisferio sur que se alimentan cerca de la Antártida en verano, pero invernan en aguas tropicales (Aguilar and García-Vernet 2018).

En el Atlántico Norte, los rorcuales comunes sí suelen realizar migraciones latitudinales, pero estas suelen ser más cortas que la de sus congéneres australes (Aguilar and García-Vernet 2018). Los datos aportados durante las últimas décadas parecen indicar que la presencia de los rorcuales es generalizada y dispersa por encima

del paralelo 32 (Nieukirk et al. 2012). Se han identificado algunas zonas concretas donde hay rorcuales comunes durante el invierno, como las Islas Azores, la zona al suroeste de Portugal o el *Purcopine Seabight*, al oeste de Irlanda (Baines et al. 2017; Pereira et al. 2020; Romagosa et al. 2020). También se han observado ballenas cruzando el Estrecho de Gibraltar en ambos sentidos (Gauffier et al. 2018), lo que sugiere que algunas de las ballenas mediterráneas pasan el invierno en el Océano Atlántico (Pereira et al. 2020) y viceversa (Castellote et al. 2012; Giménez et al. 2013). Finalmente, también se ha detectado la presencia de rorcual común durante el invierno en las zonas de veraneo (Heide-Jørgensen et al. 2003). Se ha propuesto que estos individuos son una parte de la misma población que veranea en aquel área, por ejemplo los individuos inmaduros que no necesitan ir a las zonas de reproducción (Mizroch et al. 2009; Soule and Wilcock 2013), aunque también podría tratarse de poblaciones que veranean en latitudes más altas y reemplazan a la población estival durante el invierno (Silva et al. 2019; Gauffier et al. 2020; Lydersen et al. 2020).

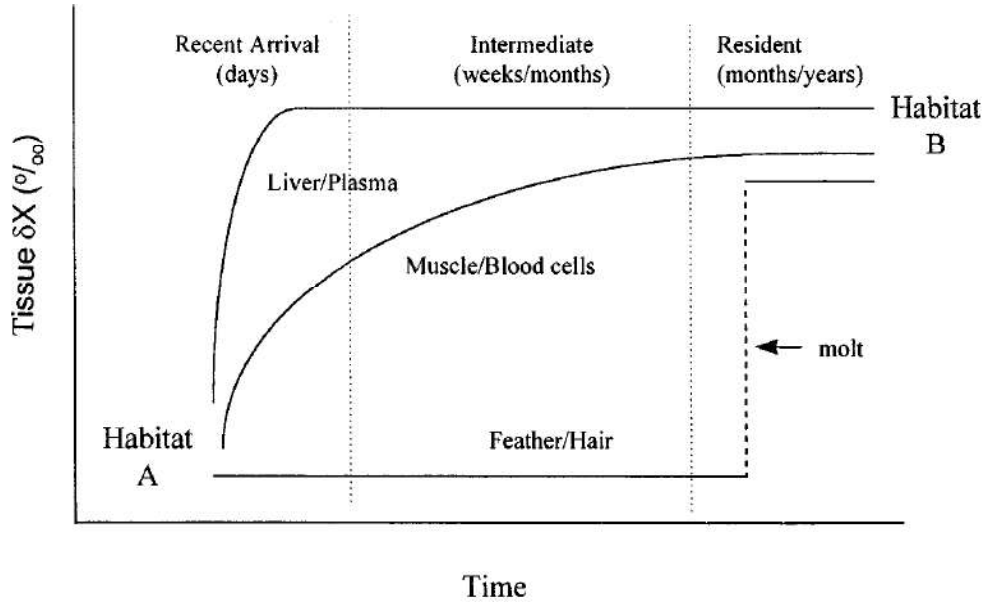


Figura 2 Cambio en los isótopos estables en tres tejidos con tasa de renovación rápida (hígado/plasma), lenta (músculo/ células sanguíneas) o metabólicamente inerte (pluma/pelo). Tras el cambio de hábitat, el plasma y el hígado adquieren la nueva señal isotópica al poco tiempo mientras que el músculo y las células sanguíneas taran un tiempo mayor. El pelo y las plumas son tejidos inertes de crecimiento limitado, así que no variarán su señal hasta el momento de la muda. Figura adaptada de Hobson (1999)

Debido a las dificultades para definir las poblaciones biológicas de los rorcuales en el Atlántico Norte, es difícil saber si los diferentes stocks utilizan las mismas regiones para invernar o si durante esa época del año se mantienen aislados (geográfica o temporalmente). Los pocos estudios que han revisado la conectividad y las rutas de migración muestran que los individuos que se alimentan en las Islas Azores en primavera se dirigen a las zonas de alimentación del este de Islandia en verano (Silva et al. 2013) y que posiblemente se habían estado alimentando cerca de la Península Ibérica durante el otoño (Silva et al. 2019; Gauffier et al. 2020). Por otro lado, parece que los individuos

Atlánticos que entran en el Mar Mediterráneo en invierno pertenecen al stock que se alimenta frente a las costas gallegas en verano (Giménez et al. 2013; Gauffier et al. 2020). Finalmente, el único estudio que marcó rorcuales comunes con marcas satelitales en las zonas de veraneo observó que la población que se alimenta cerca de las Islas de Svalbard se dispersan durante el otoño (Lydersen et al. 2020). De los rorcuales marcados, algunos individuos se quedaron en la misma zona durante el otoño, algunos se dirigieron al Mar de Noruega y a la zona que rodea Islandia, y un individuo llegó a bajar hasta la zona al suroeste de Portugal. Estos estudios parecen indicar que los individuos de un stock pueden ocupar las áreas típicamente asignadas a un stock diferente. Sin embargo, queda por saber si durante estas visitas se encuentran con individuos del stock residente, lo que permitiría el flujo genético entre stocks, o si estas visitas se producen en el momento que el stock residente ha migrado a otras áreas.

Como consecuencia de su mayor detectabilidad en verano, y que las explotaciones hayan sido fundamentalmente estivales, la dieta de los rorcuales comunes, al igual que su hábitat, ha sido mejor estudiada en verano que en invierno. Durante el verano, la mayoría de stocks del Atlántico Norte se alimenta de eufausiáceos o *krill* (Aguilar 1985; Sigurjónsson and Víkingsson 1997; Silva et al. 2013; Ryan et al. 2014), sobre todo de la especie *Meganyctiphanes norvegica*, el eufausiáceo que forma poblaciones más densas y abundantes en el Atlántico Norte (Siegel 2000). Sin embargo, durante los meses de otoño y primavera algunos grupos de rorcual común pueden alimentarse en bancos de peces. En Noruega los rorcuales comunes complementan su dieta con capelín (*Mallotus villosus*) durante la primavera y con arenque (*Clupea harengus)* durante el otoño y, posiblemente, invierno (Christensen et al. 1992). Al sur de Irlanda, los rorcuales comunes se acercan a la costa durante el otoño, donde parece que se alimentan de bancos de arenque que han ido a desovar (Whooley et al. 2011). Así pues, los rorcuales parecen tener preferencia por el krill, pero pueden complementar su dieta con peces en las épocas en que estos se agregan para la freza, que suele ser en primavera o en otoño.

Trazadores químicos

El paradigma clásico de la migración de las ballenas asume que los misticetos ayunan, o como mínimo disminuyen de una manera drástica su ingesta alimentaria durante el invierno. Es cierto que los rorcuales comunes tienen un balance nutricional negativo durante el invierno (Lockyer 1981), es decir gastan más energía de la que ingieren, pero hay múltiples pruebas que muestran que los rorcuales comunes se alimentan hasta cierto punto durante esta época. Hay observaciones de rorcuales comunes alimentándose en otoño e invierno (Christensen et al. 1992; Whooley et al. 2011; Baines et al. 2017) y su señal isotópica cambia a lo largo del año de manera diferente a como lo haría en una situación de ayuno total (Aguilar et al. 2014; Silva et al. 2019). Esto abre la posibilidad de utilizar trazadores químicos para estudiar las migraciones de los rorcuales comunes (Hobson 1999).

Los trazadores químicos son moléculas o átomos exógenos que pueden detectarse en los tejidos de los animales y cuya presencia o ausencia proporciona información sobre la localización, dieta o estado fisiológico del animal. Estos trazadores suelen ser adquiridos por el animal generalmente a través de la dieta, aunque hay algunos ejemplos de trazadores que se adsorben en los tejidos directamente del ambiente (ej. Vighi et al. 2019). Una vez introducidos, los trazadores químicos pueden quedar almacenados en los tejidos del animal en escalas de tiempo variables. En el caso de compuestos difíciles de degradar y excretar, se produce la bioacumulación: la concentración de los trazadores químicos incrementa con la edad del animal (Aguilar and Borrell 1994; Morel et al. 1998; Troisi et al. 2000). La bioacumulación dificulta el uso de estos trazadores químicos para obtener información en una escala temporal pequeña ya que la ingestión del trazador puede haberse realizado recientemente o en el pasado. Por otro lado, si el trazador químico no se acumula en los tejidos, su concentración en el animal estará en equilibrio con la concentración del medio ambiente (Vighi et al. 2017; Hobson 2019; Garcia-Garin et al. 2020). Esto significa que, si la concentración o composición del trazador en la dieta cambia, el cambio se verá reflejado en el organismo estudiado en una escala de tiempo pequeña, que dependerá de la vida media del compuesto en el organismo i/o tejido.

Los isótopos estables han sido largamente utilizados como trazadores químicos. La tasa de renovación de los tejidos que los alojan es una de las características más importantes para interpretar su utilidad como trazadores químicos (Hobson et al. 2010). En tejidos con tasa de renovación rápida, como el plasma sanguíneo, los isótopos estables proporcionan información reciente, en una escala temporal que puede ser de horas o días (Hilderbrand et al. 1998; Käkelä et al. 2005; Podlesak et al. 2005). Por otro lado, en tejidos con una tasa de renovación baja, como el colágeno del hueso, la información proporcionado por ellos suele ser un promedio de los últimos meses o años (Newsome et al. 2006). Finalmente, también existe el caso de los tejidos metabólicamente inertes. Estos tejidos fijan la señal de los isótopos estables que había en el organismo en el momento de su síntesis. Si, además, el tejido es de crecimiento continuo, cada porción del tejido contendrá los trazadores químicos correspondientes a un momento diferente de la vida del animal (Ayliffe et al. 2004; Hobson et al. 2004; Ramos and González-Solís 2012; Lübcker et al. 2016) (Figura 2). Es decir, preservan un registro temporal de los cambios en la concentración del trazador químico. En el caso de los rorcuales, hay dos tejidos especialmente útiles con este fin: los conos de cera auditivos y las barbas (Mitani et al. 2006; Robinson et al. 2013; Aguilar et al. 2014).

Isótopos estables

Los isotopos estables son aquellos isótopos de un elemento (átomos con el mismo número de protones, pero diferente número de neutrones) que no se descomponen de manera radioactiva. Las concentraciones de isótopos estables de una muestra se expresa como la razón entre el isótopo pesado y el isótopo ligero presente

en la muestra dividida por la misma ratio en una muestra estándar, menos uno (Bond and Hobson 2012):

$$\delta\ {}^{j}X = \frac{\left({}^{j}X/\ {}^{i}X\right)_{muestra}}{\left({}^{j}X/\ {}^{i}X\right)_{estándar}} - 1$$

donde X es el elemento, y los pesos atómicos del isótopo ligero y pesado vienen dados por *i* y *j* respectivamente. Las muestras estándares son producidas por el Organismo Internacional de Energía Atómica (IAEA), y son muestras con una proporción isotópica fija y conocida.

Las propiedades químicas de los isótopos de un mismo elemento son casi idénticas, por lo que los organismos pueden utilizar los diferentes isótopos de un elemento indistintamente. Sin embargo, las pequeñas diferencias en estas propiedades hacen que la proporción entre los isótopos varíe en función de las condiciones ambientales. Por ejemplo, las moléculas de agua formada por los isótopos pesados del oxígeno o el hidrógeno tienen una tasa de evaporación ligeramente menor que las formadas por los isótopos ligeros (McMahon et al. 2013a). Esto provoca que en aquellas regiones cálidas donde hay mucha evaporación de agua la proporción de isótopos pesados de oxígeno e hidrógeno sea mayor (McMahon et al. 2013b).

En biología, los isótopos estables más utilizados son los del carbono ($\delta^{13}C$) y del nitrógeno ($\delta^{15}N$). Las fuentes de variación son similares en los dos casos, aunque cada uno se ve afectado en mayor o menor medida por las distintas fuentes. En ambos casos la variación ambiental es una fuente de variación muy importante (McMahon et al. 2013b). La señal isotópica ambiental varía latitudinalmente, siendo el ^{13}C más enriquecido en las latitudes ecuatoriales y el ^{15}N más enriquecido en las zonas boreales. A pequeña escala, la señal isotópica ambiental también puede variar. La señal de $\delta^{13}C$ se ve afectada por la distancia a la costa (Hobson et al. 1994) mientras que la señal de $\delta^{15}N$ puede indicar zonas de emergencia de agua profunda rica en nitratos (Mahaffey et al. 2008). La variación geográfica y oceanográfica de la señal isotópica ambiental permite el uso de estos isótopos estables como indicadores de las zonas de alimentación (**¡Error! No se encuentra el origen de la referencia.**).

La segunda fuente de variación en los organismos es su dieta. Si bien los isótopos estables tienen propiedades casi idénticas, las enzimas suelen tener preferencia por unos de los isótopos, generalmente el isótopo ligero. Así pues, en cada proceso enzimático se seleccionará uno de los isótopos por encima del otro, lo que se conoce como discriminación isotópica (Hobson et al. 2010). En el carbono, la discriminación isotópica es especialmente importante en dos casos: durante la fijación del carbono (Collister et al. 1994) y en la síntesis de los ácidos grasos (Post et al. 2007; Caut et al. 2011). En el primer caso, diferentes productores primarios discriminarán el carbono de manera diferente, por lo que la señal de $\delta^{13}C$ en los organismos puede ser usada como

un marcador de la fuente de carbono. En el segundo caso, la señal de $\delta^{13}C$ podría ser usada para conocer la concentración de ácidos grasos de un tejido. Sin embargo, debido a que la presencia de esta fuente de variación dificulta la interpretación de los datos, los lípidos suelen ser extraídos del tejido antes del análisis isotópico. La señal de $\delta^{15}N$ también se ve afectada por procesos enzimáticos. En este caso, la discriminación isotópica principal se da en la desaminación y transaminación de los aminoácidos (Macko et al. 1987). Esto hace que, durante la excreción del nitrógeno, el nitrógeno ligero sea eliminado del organismo en mayor proporción que el isótopo pesado. En consecuencia, la señal isotópica del organismo queda "enriquecida" de nitrógeno pesado. Debido a esto, los animales suelen tener una señal de $\delta^{15}N$ superior a la de su dieta. El incremento de la señal $\delta^{15}N$ entre la dieta y los tejidos del animal es relativamente constante en todos los niveles tróficos, por lo que este isótopo estable suele utilizarse para estimar el nivel trófico del animal (Post 2002).

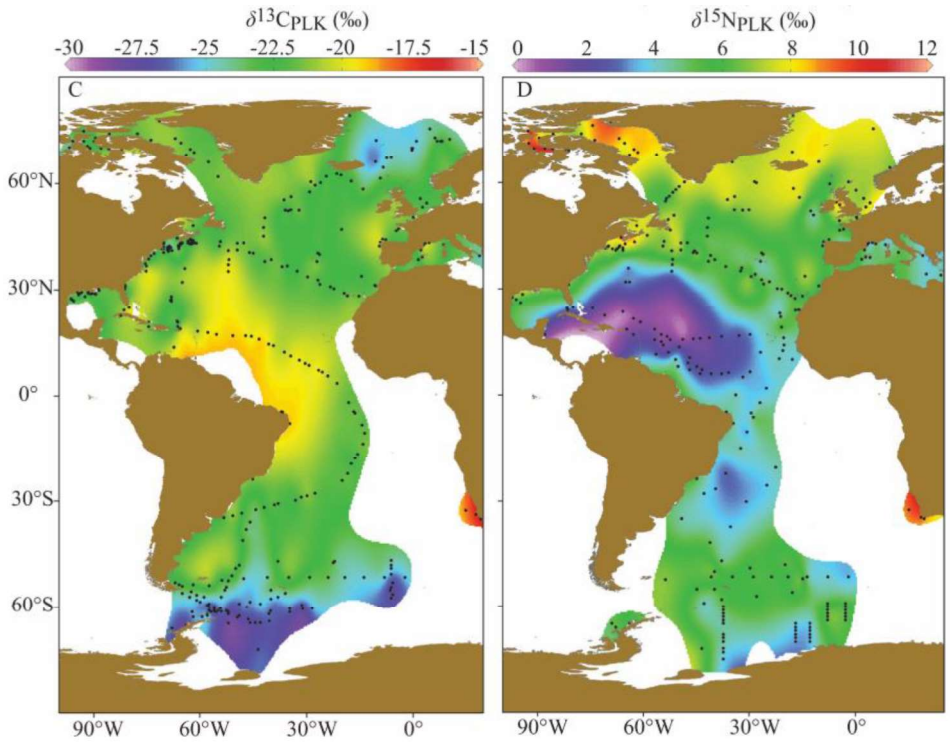


Figura 3 Variación geográfica de los isótopos estables del carbono (izquierda) y nitrógeno (derecha) detectados en muestras de plancton. Figura adaptada de McMahon 2013b.

Finalmente, otra fuente de variación de los isótopos estables es la fisiología del animal. Cambios del estado reproductor, ayunos o épocas de crecimiento son algunos de los factores que pueden hacer variar las señales de $\delta^{13}C$ y $\delta^{15}N$, aunque el grado de variación depende en gran medida del tejido y de la especie analizada (Warinner and Tuross 2010; Borrell et al. 2016).

Debido a las múltiples fuentes de variación de los isótopos estables, y a la dificultad en diferenciarlas, se ha desarrollado una técnica que analiza los isótopos estables del nitrógeno y del carbono en moléculas específicas (CSIA por sus siglas en

inglés). En concreto, el análisis de la señal de $\delta^{15}N$ en aminoácidos concretos (CSIA-AA) es una técnica cada vez más utilizada para diferenciar los efectos de las diferentes fuentes de variación (Matthews and Ferguson 2013; Ruiz-Cooley et al. 2014; Brault et al. 2019). Debido a que cada aminoácido tiene un metabolismo diferente dentro del cuerpo, los isótopos que los componen se verán discriminados de manera diferente. Por ejemplo, la fenilalanina es un aminoácido esencial en los animales (Costa et al. 2014) y sufre una discriminación mínima, por lo que sirve como indicador de la señal isotópica ambiental (McClelland and Montoya 2002; Popp et al. 2007). Por otro lado, el glutamato/glutamina (durante el proceso analítico ambos aminoácidos forman el mismo producto y se analizan conjuntamente) actúa como reservorio de nitrógeno en el cuerpo por lo que se verá muy afectado por la discriminación producida durante la transaminación (McClelland and Montoya 2002; Popp et al. 2007). Los aminoácidos que mantienen la señal isotópica en todos los niveles tróficos se los conoce como aminoácidos "fuente" mientras que los que sufren discriminación isotópica se los conoce como "tróficos" (Popp et al. 2007); la diferencia entre la señal isotópica de los aminoácidos de estos dos grupos puede usarse para calcular de manera precisa el nivel trófico (Chikaraishi et al. 2009).

Alquenonas

Las alquenonas son un grupo de moléculas producidas por un grupo de organismos haptófitos muy utilizadas para estimar la temperatura del agua. Las dos moléculas más utilizadas son la $C_{37:2}$ y la $C_{37:3}$, que se componen de una cadena de 37 carbonos con un grupo cetónico y dos o tres insaturaciones, respectivamente. La proporción de $C_{37:2}$ respecto a la suma de las dos se conoce como el índice $U^{k'}_{37}$ (Prahl et al. 1988) y está muy correlacionada con la temperatura del agua en la que viven los haptófitos (Prahl and Wakeham 1987; Conte et al. 2006). Las moléculas, que son extremadamente resistentes, sedimentan en el fondo marino y esto ha hecho que hayan sido utilizadas por los paleo-oceanógrafos para estudiar la temperatura en el pasado (McCaffrey et al. 1990; Brassell et al. 2004; Bendle and Rosell-Melé 2007). Estas moléculas también pueden transmitirse por la cadena trófica y proporcionan información del hábitat donde se alimenta la especie analizada (Rita et al. 2021). Si bien las alquenonas prácticamente no han sido analizadas en tejidos animales, su resistencia a la degradación y su relación con la temperatura ambiental las convierten en candidatas ideales a ser utilizadas como marcadores químicos de temperatura. Estas moléculas serían especialmente útiles para estudiar la temperatura a la que se alimenta una población cuando las zonas de alimentación sean desconocidas.

Tejidos estudiados

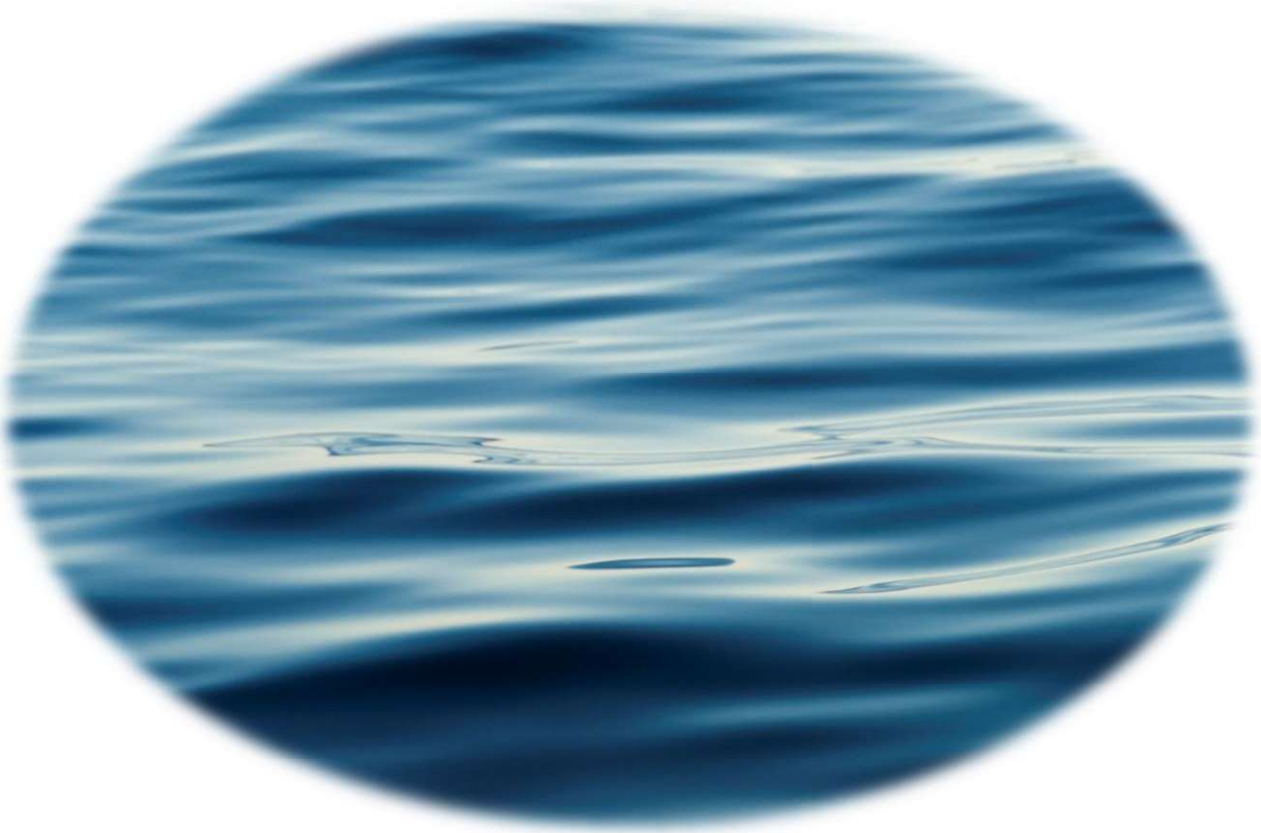
En cada capítulo de la presente tesis se ha analizado un tejido de rorcual común diferente para obtener información sobre las migraciones de esta especie en tres escalas temporales diferentes. Los tejidos estudiados fueron: la grasa hipodérmica, la barba y el cono de cera auditivo. Cada capítulo proporciona una descripción más detallada del

tejido en el que se basa, pero a continuación se resumen las propiedades de los tres tejidos.

La grasa hipodérmica es un tejido con alto contenido lipídico y diferente al tejido adiposo que usualmente se halla presente en el del resto de los mamíferos. Este tejido, además de actuar como una reserva de energía, tiene funciones metabólicas, es térmicamente aislante y, gracias a su alto contenido en proteínas fibrosas, también tiene funciones estructurales (Galligan et al. 2018; Iverson and Koopman 2018). Las alquenonas, moléculas lipofílicas sin efectos biológicos fuera del grupo de los haptófitos, pueden ser almacenadas temporalmente en la grasa hipodérmica de las ballenas. Esto permite obtener información relacionada con la temperatura del agua en la que se alimentó el individuo. Por desgracia, la novedad de la técnica implica que exista un desconocimiento importante sobre la tasa de renovación de las alquenonas en la grasa hipodérmica, lo que dificultará la interpretación de los resultados.

Las barbas son láminas triangulares flexibles de queratina sintetizadas en la encía (Rice 2002). Las barbas penden del maxilar superior de las ballenas formando el aparato filtrador que sirve a estos animales para alimentarse. Las barbas de rorcual común miden unos 60 cm y crecen a razón de ~20cm al año (Aguilar et al. 2014); es decir, contienen un registro de unos 3 años de la señal isotópica del animal. Las barbas están formadas por dos tejidos diferentes: una medula central formada por túbulos y una corteza que rodea la médula por los dos lados (Fudge et al. 2009). La corteza se erosiona con el tiempo y cuando desaparece deja al descubierto los túbulos, los cuales actúan como unos filamentos que se entrecruzan, formando el filtro que permite capturar el alimento.

Por último, los conos de cera son estructuras formadas en los canales auditivos de los misticetos. Están formados por células epiteliales que se acumulan a lo largo de toda la vida del individuo (Roe 1967). Gracias a que el tejido formado en verano tiene una composición y un color diferentes al formado en invierno, en ellos se pueden distinguir bandas de crecimiento formadas anualmente a lo largo de la vida del animal (Laws and Purves 1956; Lockyer 1984).



Objetivos

Objetivos

El objetivo global de esta tesis doctoral es conocer mejor los patrones de migración del rorcual común y su biología durante el invierno. En concreto, los objetivos específicos que se espera alcanzar o preguntas que se espera responder son:

–¿Qué tipo de hábitat utilizan los rorcuales comunes durante el invierno?

–¿De qué se alimentan durante el invierno?

–¿Son sus migraciones consistentes temporalmente, o cambian cada año?

Para poder contestar estas preguntas se analizaron tres tejidos diferentes, cuyas características permiten obtener información a escalas temporales diferentes. La información obtenida en estas escalas temporales se combinará en la discusión para responder estas preguntas y así proporcionar un mejor marco de conocimiento de las migraciones del rorcual común.

Capítulo 1

Durante el capítulo 1 se analizarán las alquenonas en los rorcuales para obtener información relacionada con la temperatura del agua en la que se alimentan durante el verano y el invierno. Dada la novedad de este tipo de análisis no se pudo obtener la temperatura exacta del agua en las zonas de invernada, pero sí se han utilizado estos datos de manera relativa para comparar el comportamiento de diferentes individuos de una o más poblaciones.

Dadas las numerosas incógnitas acerca de la aplicación de estos marcadores en mamíferos marinos, se realizó, como paso previo, un estudio dedicado a conocer en qué tejidos del rorcual común pueden encontrarse las alquenonas. Una vez se obtuvo esta información se realizó un segundo estudio más amplio con el objetivo de obtener información sobre las zonas donde habían habitado recientemente los rorcuales.

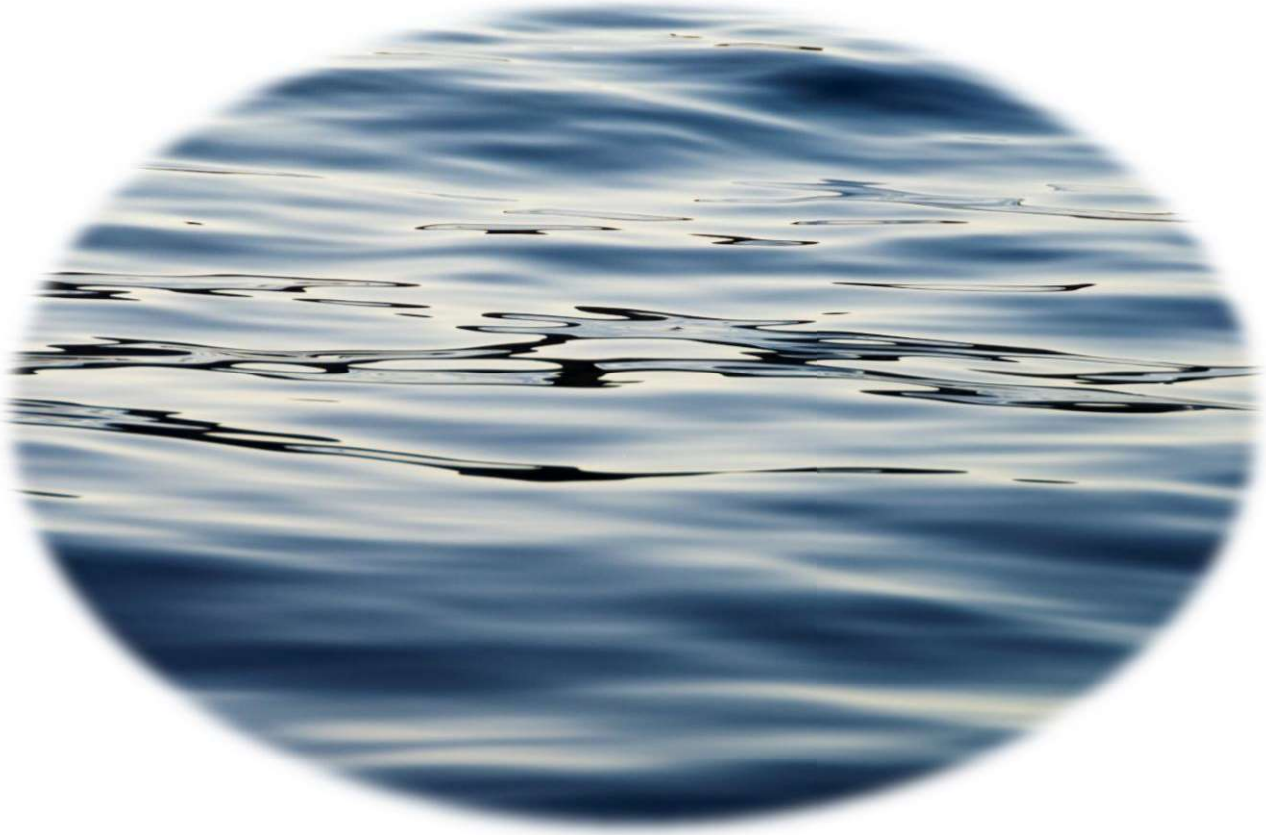
Capítulo 2

El objetivo del segundo capítulo fue comparar los valores isotópicos ambientales y el nivel trófico de los rorcuales comunes en las zonas de veraneo y de invernada. Para ello se analizaron los isótopos estables del nitrógeno en aminoácidos específicos en muestras de barba de ballena sintetizada durante el verano y en muestras sintetizadas durante el invierno.

Para poder realizar el muestreo correctamente fue preciso hacer un análisis histológico previo con el objetivo de determinar las diferencias en la síntesis de los dos tejidos que forman la barba de ballena y seleccionar el tejido más adecuado para los análisis de CSIA-AA.

Capítulo 3

El tercer capítulo está dedicado a estudiar la consistencia temporal de las migraciones del rorcual común. Para ello se analizaron los isótopos estables del carbono y del nitrógeno en muestras de conos de cera. La información obtenida permite calcular la especialización individual en esta población y la consistencia temporal de esta especialización individual.



Informe de los Directores

Informe de los Directores

Como directores de la tesis doctoral titulada "Estructura y migración del rorcual común del Atlántico nororiental establecido mediante trazadores químicos" realizada por Diego Rita Espada, presentamos el siguiente informe sobre la contribución del doctorando en las publicaciones en coautoría ya realizadas que componen la tesis:

Capítulo 1. Rita, D.; Borrell, A.; Berdié, L.; Aguilar, A. 2020 Alkenones as a temperature proxy in fin whale (Balaenoptera physalus) tissues Limnol Oceanogr-Meth; DOI: 10.1002/lom3.10375

Contribución del doctorando: Participación en el diseño de los experimentos, desarrollo de la técnica de análisis químico, realización de los análisis químicos y estadísticos y redacción del manuscrito.

Acerca de la revista: *Limnology and Oceanography: Methods.* En el Journal Citation Reports (JRC) de 2019 tiene un índice de impacto de 2.46. Se encuentra en la posición 4 de 22 en el área de Limnología (1er cuartil) y en la posición 24 de 67 en el área de oceanografía (2º cuartil).

Capítulo 2. – Rita. D.; Borrell, A.; Víkingsson, G.; Aguilar, A. 2019 Histological structure of baleen plates and its relevance to sampling for stable isotope studies. J. Mamm Biol. 99:63-70; DOI: 10.1016/j.mambio.2019.10.004

Contribución del doctorando: Participación en el diseño de los experimentos, realización de los cortes histológicos y tinción de las muestras, análisis de isótopos estables y estadísticos, y redacción del manuscrito.

Acerca de la revista: *Journal of Mammalian Biology*. En el Journal Citation Reports (JRC) de 2019 tiene un índice de impacto de 1.595. Se encuentra en la posición 54 de 169 en el área de Zoología (2º cuartil)

Los otros tres trabajos en preparación serán enviados a publicar en un futuro

El manuscrito "Alkenone $U^{k'}_{37}$ index differs between thermally separated populations of fin whales and krill" será enviado a la revista *Limnology And Oceanography*, que tiene un índice de impacto de 3.77 y se encuentra en la posición 3 de 22 del área de Limnologia (1er cuartil) y la posición 6 de 67 del área de Oceanografía (1er cuartil).

El manuscrito "Amino acid-specific nitrogen stable isotope analysis reveals the winter biology of Icelandic fin whales" será enviado a la revista Marine Mammal Science,

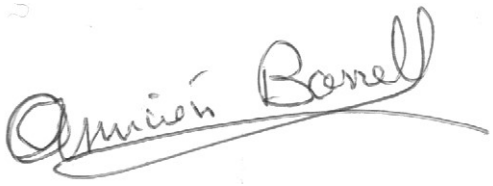
que tiene un índice de impacto de 1.65 y se encuentra en la posición 45 de 107 en el área de Biología Marina y de Agua Dulce y la posición 53 de 169 del área de Zoología.

El manuscrito "Earplugs provide insight into possible specialisation in fin whales (*Balaenoptera physalus*)" será enviado a la revista *Marine Ecology Progress Series*, que tiene un índice de impacto de 2.33 y se encuentra en la posición 72 de 169 del área de Ecología, la posición 26 de 107 de área de Biología Marina y de Agua Dulce y la posición 27 de 67 del área de Oceanografía.

Barcelona, a 21 de abril de 2021

Firma de los directores

Dr. Alejandro Aguilar Vila
Departamento de Biología Evolutiva, Ecología y Ciencias Ambientales
Facultad de Biología



Dra Assumpció Borrell Thió
Departamento de Biología Evolutiva, Ecología y Ciencias Ambientales
Facultad de Biología

Capítulo 1

Alkenones as a temperature proxy in fin whale (*Balaenoptera physalus*) tissues

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Estado de publicación: publicado en julio del 2020 en *Limnology and Oceanography: Methods*

Abstract: Alkenones are a group of extremely resilient molecules produced by cosmopolitan haptophyte organisms. The unsaturation index ($U^{k'}_{37}$) of di- vs. tri-unsaturated C_{37} alkenones ($C_{37:2}$ ($C_{37:2}$ + $C_{37:3}$)$^{-1}$) can be used to estimate the temperature of the water in which the alkenone-producing organisms grew. Alkenones have been widely used in paleoceanography, but they have received little attention in other fields. In this study, a method to detect alkenones in fin whale (Balaenoptera physalus) tissues is developed to adapt this technique to the marine ecology field. Five replicas of five tissues (stomach content, external blubber, internal blubber, muscle, and liver) were analyzed using gas chromatography coupled to mass spectrometry. Alkenones were present in both blubber tissues (66 ± 57 ng g^{-1} in external blubber and 145 ± 89 ng g^{-1} in internal blubber), and in the stomach content (3126 ± 2643 ng g^{-1}). The calculated $U^{k'}_{37}$ index was very similar in the three tissues: 0.54 ± 0.03 in the external blubber, 0.55 ± 0.08 in the internal blubber, and 0.71 ± 0.06 in the stomach content. These indexes are equivalent to a sea surface temperature estimates of 17.79 ± 0.68oC in the external blubber, 17.84 ± 1.84°C in the internal blubber, and 21.07 ± 1.23°C in the stomach content, which are very similar to the expected temperature for the region. The results of the current study indicate that alkenones biodilute in the trophic web, which could hinder the analyses of alkenones in species with a high trophic level. However, it is shown that alkenones can be detected in fin whale tissues and can be used to approximate the environmental water temperature where these animals feed.

LIMNOLOGY and OCEANOGRAPHY: METHODS

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Alkenones as a temperature proxy in fin whale (*Balaenoptera physalus*) tissues

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Abstract

Alkenones are a group of extremely resilient molecules produced by cosmopolitan haptophyte organisms. The unsaturation index ($U^{k'}_{37}$) of di- vs. tri-unsaturated C_{37} alkenones ($C_{37:2}\cdot(C_{37:2}+C_{37:3})^{-1}$) can be used to estimate the temperature of the water in which the alkenone-producing organisms grew. Alkenones have been widely used in paleoceanography, but they have received little attention in other fields. In this study, a method to detect alkenones in fin whale (*Balaenoptera physalus*) tissues is developed to adapt this technique to the marine ecology field. Five replicas of five tissues (stomach content, external blubber, internal blubber, muscle, and liver) were analyzed using gas chromatography coupled to mass spectrometry. Alkenones were present in both blubber tissues ($66 \pm 57\ ng\ g^{-1}$ in external blubber and $145 \pm 89\ ng\ g^{-1}$ in internal blubber), and in the stomach content ($3126 \pm 2643\ ng\ g^{-1}$). The calculated $U^{k'}_{37}$ index was very similar in the three tissues: 0.54 ± 0.03 in the external blubber, 0.55 ± 0.08 in the internal blubber, and 0.71 ± 0.06 in the stomach content. These indexes are equivalent to a sea surface temperature estimates of $17.79 \pm 0.68°C$ in the external blubber, $17.84 \pm 1.84°C$ in the internal blubber, and $21.07 \pm 1.23°C$ in the stomach content, which are very similar to the expected temperature for the region. The results of the current study indicate that alkenones biodilute in the trophic web, which could hinder the analyses of alkenones in species with a high trophic level. However, it is shown that alkenones can be detected in fin whale tissues and can be used to approximate the environmental water temperature where these animals feed.

Chemical tracers in organisms have been widely used to obtain ecological information. The analysis of these compounds in animal tissues can provide diverse individual information, such as diet (Cardona et al. 2012), habitat (Bayes et al. 2014) and physiological state (Hobson et al. 1993; Borrell et al. 2016). However, the environmental temperature of the waters where an organism lives cannot currently be investigated through chemical tracers but can be studied only through thermometers or through remote sensing using satellites. As adequate water temperature is crucial for marine mammals' wellness (Rasmussen et al. 2007; McIntyre et al. 2011; Owen et al. 2018), new techniques to measure temperature are valuable, particularly in situations where the temperature at which animals had been exposed remains unknown, as is the case for stranded or museum specimens.

The analysis of alkenones, which has been widely used in paleoceanography, is an effective tool to measure environmental water temperature (e.g., McCaffrey et al. 1990; Kennedy and Brassell 1992; Brassell et al. 2004; Bendle and Rosell-Melé 2007; Lourenço et al. 2016; Sánchez-Montes et al. 2020). Alkenones are highly resilient molecules composed of a group of di-, tri-, and tetra-unsaturated long-chain ketones, (de Leeuw et al. 1980) that remain in the sediments for millions of years with little degradation (Brassell et al. 2004). They can be 37, 38, or 39 carbon long ketones, but the most abundant and commonly used are the 37 carbon long (C37). Alkenones are exclusively produced by a group of haptophyte organisms, such as *Emiliania huxleyi* (Marlowe et al. 1984), which, depending on the water temperature, generate di- ($C_{37:2}$) and tri-unsaturated ($C_{37:3}$) alkenones in different proportions. This characteristic led to the development of the index $U^{k'}_{37}$ ($[C_{37:2}]/([C_{37:2}]+[C_{37:3}]$) (Prahl et al. 1988). Previous alkenone indexes, that is, U^{k}_{37}, had been defied taking into consideration the tetra-unsaturated C37 alkenones ($C_{37:4}$) (Brassell et al. 1986*a*,*b*) However, since the $C_{37:4}$ is present only in freshwater or polar ocean and can be difficult to detect

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in those samples obtained in temperate and tropical waters, we used the $U^{k'}_{37}$. The $U^{k'}_{37}$ index ranges between 0 (when only $C_{37:3}$ is present in the environment) and 1 (when only $C_{37:2}$ is present) at extremely cold or warm temperatures, respectively; moreover, it strongly correlates with the sea surface temperature range of most oceans, providing a reliable temperature estimation (Conte et al. 2006). Therefore, alkenones in oceanic sediment cores have been used by paleoceanographers to estimate the water temperature from millions of years ago (e.g., Sachs and Lehman 1999; Brassell et al. 2004; Caissie et al. 2010; Nieto-Moreno et al. 2013).

Whether alkenones can be assimilated by vertebrate animals is, so far, an open question. Only a few studies have analyzed alkenones in invertebrate animal tissues and showed that the assimilation rate was very low (Volkman et al. 1980; Rowland and Volkman 1982; Grice et al. 1998). However, alkenones were present in their digestive tract and their pellets, thus, opening the possibility of vertebrate carnivores ingesting alkenones indirectly. If alkenones could be detected in vertebrate tissues, the $U^{k'}_{37}$ index could be used to measure the environmental water temperature of the feeding habitats of these species. Furthermore, this temperature proxy in animals might be used to develop a new set of studies in marine ecology.

The aim of the present study was to develop a method to detect alkenones in fin whale (*Balaenoptera physalus*) tissues and in its prey (*Meganyctiphanes norvegica*) (Aguilar 1985) in order to adapt this temperature proxy to the marine ecology field. The secondary objective of the current study was to investigate whether fin whales showed higher (biomagnification) or lower (biodilution) concentrations of alkenones than their prey.

The studied fin whale population alternates between the summer feeding ground, near the north-west coast of Spain, and the winter breeding grounds at low latitudes (Aguilar and García-Vernet 2018). In the feeding grounds, this population feed mainly on krill, which may facilitate the ingestion of alkenones. Although zooplankton does not seem to assimilate alkenones, some species may feed on the haptophyte species that synthesize them (Volkman et al. 1980; Grice et al. 1998). Thus, fin whales have the potential to ingest the alkenones present in the krill's stomach (indirect intake) or from the other organisms also present in the krill shoal.

Materials and procedures

Alkenones were analyzed in blubber, muscle, and liver tissues and in the stomach content (krill) of five fin whales (Table 1). Because of the heterogeneity of blubber throughout its depth (Lockyer et al. 1984; Aguilar and Borrell 1990), two distinctive zones were analyzed: external blubber (under the skin) and internal blubber (adjacent to the muscle). All samples were collected from fin whales caught during commercial whaling operations in northwest Spain in 1984 and 1985 and were kept frozen at −20°C until analysis. To test the reproducibility of the method, the same sample (i.e., the internal blubber of the fin whale 84001) was analyzed 10 times.

Prior to analysis, samples were freeze-dried for 48 h. Then, liver, muscle, and stomach content were ground into powder, and blubber samples were cut into small pieces. Approximately 1 g dry weight of each sample was mixed with 50 μL of the internal standard (2-pentatriacontanone; 20 ng μL^{-1} in n-hexane) in a centrifuge tube for the alkenone analysis.

Samples were then saponified in 4 mL of 3 mol L^{-1} KOH in water (H_2O) : methanol (MeOH) 1 : 9 (v/v) for 60 min at 80°C in an ultrasound bath. Afterward, 4 mL of n-hexane was added, and the centrifuge tubes were vortexed for 1 min and centrifuged at 5000 rpm for 4 min. The n-hexane phase, which contained the nonsaponifiable lipids, was transferred to another centrifuge tube. This n-hexane extraction was repeated three times. The extracted n-hexane was combined with 12 mL of 3 mol L^{-1} KOH in H_2O, and the centrifuge tubes were vortexed once more for 1 min and centrifuged for 15 min at 5000 rpm. The n-hexane phase was extracted again and passed through Na_2SO_4 into a pear flask. Then, 12 mL of n-hexane was added again into the KOH/H_2O vials to remove any nonsaponifiable lipid left. The vials were vortexed and centrifuged one last time, and the n-hexane was also passed through the Na_2SO_4 into the pear flasks. The pear flasks were placed in a rotary evaporator, and the n-hexane was evaporated down to 1–2 mL. This 1–2 mL of nonsaponifiable lipid extract was further purified using solid-phase extraction (Supelclean LC-NH_2 SPE tubes; 3 mL). The first fraction, eluted with 4 mL of n-hexane, contained the hydrocarbon fraction and was discarded. The second fraction, eluted with 6 mL of n-hexane : dichloromethane 3 : 1 (v/v), contained the ketone fraction (Leider et al. 2010). This fraction was dried under N_2 stream and solved in 20 μL of n-hexane prior to analysis.

Gas chromatography–Mass spectrometry was performed using a gas chromatograph (Shimadzu GCMS-QP2010) equipped with a 30 m Sapiens-X5MS silica capillary column (0.25 mm internal diameter, 0.25 μm film thickness) and a mass spectrometer detector (a quadrupole working in the electronic ionization mode), using He as a carrier gas with a flow rate of 1 mL min^{-1}. The GC temperature programme was as follows: injection at 60°C; 1 min isothermal; 60–310°C at 40°C min^{-1}; 28 min isothermal with a total run-time of 36 min. Injection temperature was 320°C and injection mode was splitless with a sampling time of 1 min. Interface temperature was 310°C and Ion Source temperature was 200°C. Acquisition was made in the scan mode with a mass range from 50 to 550 amu. Peak identification of C_{37} alkenones was based on retention time and the comparison of the ion spectrum with those of pure alkenone standards.

The concentrations of both alkenones were quantified using the area of the ion with m/z 81. This ion was used because it provided a higher signal-to-noise ratio than the total ion composition. Pure alkenone standards, kindly

Table 1 Summary of the whale samples analyzed in this study.

Individual	Year	Liver	Muscle	Internal blubber	External blubber	Stomach content
85021	1985					+
85022	1985					+
85027	1985					+
85030	1985					+
85041	1985					+
84001	1984	+	+	+ (x10)	+	
84023	1984	+	+	+	+	
84046	1984	+	+	+	+	
84051	1984	+	+	+	+	
84054	1984	+	+	+	+	

provided by David Naafs from Bristol University, were used to measure the response factor of m/z 81 of each alkenone, and the internal standard was used for quantification. The detection limit of this method was 9 ng g^{-1}.

$U^{k'}_{37}$ was calculated as

$$U^{k'}_{37} = \frac{[C_{37:2}]}{[C_{37:2}] + [C_{37:3}]}$$

where $[C_{37:2}]$ and $[C_{37:3}]$ are the concentrations of each alkenone in the sample (Prahl et al. 1988). $U^{k'}_{37}$ was later transformed into temperature (T) using the Conte et al. (2006) equation for the Atlantic region:

$$T = 48.673\left(U^{k'}_{37}\right)^3 - 94.569\left(U^{k'}_{37}\right)^2 + 80.716\left(U^{k'}_{37}\right) - 5.977$$

Assessment

$C_{37:2}$ and $C_{37:3}$ alkenones were found in all the stomach content and internal blubber samples and in four of the five external blubber samples. $C_{37:4}$, C_{38}, and C_{39} alkenones were not detected in any sample. The stomach content showed the highest alkenone concentration (3126 ± 2643 ng $\cdot g^{-1}_{dw}$; mean ± standard deviation [SD]). The external blubber contained 66 ± 57 ng $\cdot g^{-1}_{dw}$, and the internal blubber contained 145 ± 89 ng $\cdot g^{-1}_{dw}$ (Table 2). The $U^{k'}_{37}$ values averaged 0.71 ± 0.06 in the stomach content, 0.54 ± 0.03 in the external blubber, and 0.55 ± 0.08 in the internal blubber, which correspond to temperatures of 21.07 ± 1.23°C, 17.79 ± 0.68°C, and 17.84 ± 1.84°C, respectively.

The reproducibility test showed that the internal blubber of whale 84001 had an alkenone concentration of 110 ± 55 ng $\cdot g^{-1}_{dw}$ (Table 3). The $U^{k'}_{37}$ of this sample was 0.63 ± 0.04, which corresponds to an environmental temperature of 19.46 ± 0.89°C.

Discussion

The reproducibility test, composed of 10 subsamples, showed that the method developed produces high variability in the alkenone concentration. Such low precision may hide trends in the alkenone concentrations and may hinder further studies of the bioaccumulation and turnover of these molecules. However, the $U^{k'}_{37}$ measurements were quite precise within the standard error of $U^{k'}_{37}$ measurements in sediment (Rosell-Melé et al. 2001), which validates the use of this method to estimate environmental water temperature. Although the relationship between $U^{k'}_{37}$ and temperature is almost lineal through the whole range, this is not the case in the extreme values (i.e., $U^{k'}_{37}$ values close to 1 and to 0). This means that the SD of the temperature will be higher for $U^{k'}_{37}$ values close to 0 or 1. For example, a $U^{k'}_{37}$ of 0.1 ± 0.04 corresponds to a temperature of 1.7 ± 2.8°C. Nevertheless, the precision would still be quite high for most water temperatures within the range of our oceans.

The presence of alkenones in the whale blubber confirmed that the fin whales had absorbed and stored the ingested alkenones, at least temporarily. The alkenone concentration in the stomach content was several orders of magnitude lower than the concentration detected in copepod pellets (8×10^5 ng g^{-1}) (Volkman et al. 1980). This was unsurprising since Volkman et al. (1980) conducted their experiments in a control environment where the copepods were fed exclusively with *E. huxleyi*. The *M. norvegica* analyzed in this study had probably fed on multiple algal and animal species. The alkenone concentration in the blubber was an order of magnitude lower than that in the stomach content, which suggests that the alkenones biodilute through the trophic web. This fact has important implications for future studies since it means that the alkenone concentration may be lower and therefore harder to detect in high trophic level species.

Blubber tissue is mainly composed of lipidic molecules, especially saturated and monounsaturated fatty acids. The

Table 2 Alkenone concentration in the five analyzed tissues. The results of the internal blubber of whale 84001 are the average (± SD) of the 10 replicas. Those samples were no alkenones were detected have been assigned a concentration bellow the detection level (< 9 ng g^{-1}).

Tissue	Whale number	$C_{37:3}$ (ng g^{-1})	$C_{37:2}$ (ng g^{-1})	Total (ng g^{-1})	U^{k}_{37}
Stomach content	85041	826.5	1990.0	2816.5	0.71
Stomach content	85021	1590.5	2611.1	4201.6	0.62
Stomach content	85022	39.1	130.2	169.3	0.77
Stomach content	85027	456.8	976.8	1433.6	0.68
Stomach content	85030	1664.7	5345.7	7010.4	0.76
Liver	84001	<9	<9	<9	
Liver	84023	<9	<9	<9	
Liver	84046	<9	<9	<9	
Liver	84051	<9	<9	<9	
Liver	84054	<9	<9	<9	
Muscle	84001	<9	<9	<9	
Muscle	84023	<9	<9	<9	
Muscle	84046	<9	<9	<9	
Muscle	84051	<9	<9	<9	
Muscle	84054	<9	<9	<9	
External blubber	84001	<9	12.8	12.8	
External blubber	84023	<9	<9	<9	
External blubber	84046	28.9	34.3	63.2	0.54
External blubber	84051	72.4	76.3	148.7	0.51
External blubber	84054	15.4	21.1	36.5	0.58
Internal blubber	84001	41.1 ± 21.1	69.7 ± 35.3	110.8 ± 55.2	0.63 ± 0.04
Internal blubber	84023	39.4	30.2	69.5	0.43
Internal blubber	84046	139.1	154.2	293.4	0.53
Internal blubber	84051	55.6	78.2	133.9	0.58
Internal blubber	84054	36.3	43.0	79.3	0.54

Table 3 Alkenone concentration in the reproducibility test.

Tissue	Whale number	$C_{37:3}$ (ng g^{-1})	$C_{37:2}$ (ng g^{-1})	Total (ng g^{-1})	$U^{k'}_{37}$
Internal blubber	84001	50.6	101.9	152.5	0.67
Internal blubber	84001	37.9	90.7	128.6	0.71
Internal blubber	84001	46.4	69.8	116.1	0.60
Internal blubber	84001	47.7	84.0	131.7	0.64
Internal blubber	84001	12.1	21.3	33.5	0.64
Internal blubber	84001	11.5	19.1	30.6	0.62
Internal blubber	84001	32.5	42.5	75.0	0.57
Internal blubber	84001	59.8	75.9	135.7	0.56
Internal blubber	84001	31.2	60.6	91.8	0.66
Internal blubber	84001	81.2	130.9	212.2	0.62
	Average ± SD	41.1 ± 21.1	69.7 ± 35.3	110.8 ± 55.2	0.63 ± 0.04

lipophilic nature of this tissue facilitates the absorption of other lipophilic molecules, such as organic pollutants (Aguilar and Borrell 1991), steroid hormones (Kellar et al. 2014; Carone et al. 2019; Cates et al. 2019), and nonsaponifiable lipids (Ackman et al. 1965). This could explain why alkenones are found in the blubber but not in other tissues. Also, mysticete blubber is not an homogenous tissue, but it stratifies in three different layers, which have different functions and compositions (Iverson and Koopman 2018). The outer layer has the highest lipid content (Aguilar and Borrell 1990) while the

inner layer tends to have higher nonsaponifiable lipid concentrations than the other two (Ackman et al. 1965). Furthermore, while the external blubber has a stable lipid content over time, the internal blubber has an active metabolic role and acts as energy storage tissue (Aguilar and Borrell 1990). Therefore, its composition is dynamic and varies depending on the reproductive and nutrition state of the individual (Aguilar and Borrell 1990). This may produce differences in the concentration of different lipid soluble compounds between the internal and the external blubber (Ackman et al. 1965; Aguilar and Borrell 1991). In the case of alkenones, the highest concentration was detected in the internal blubber, which suggests that alkenones may be subject to the same dynamic variations as other compounds in the blubber layers.

The sea temperature estimated from the stomach content samples was quite similar to the temperatures recorded at the fin whale feeding ground, near the north-west coast of Spain (19.9 ± 0.6°C) (Reynolds and Stokes 1981). The $U^{k'}_{37}$ and therefore the estimated sea temperature in the two blubber layers was lower and more variable than in the stomach content. This difference could have two potential explanations. On the one hand, the stomach content samples were collected in 1985, which was slightly warmer than 1984 (Reynolds and Stokes 1981), the year when the blubber samples were collected. On the other hand, the $U^{k'}_{37}$ measured in the blubber layer could be integrating a longer period than the $U^{k'}_{37}$ measured in the stomach content. In the latter case, fin whales would have to bioaccumulate the alkenones (i.e., alkenones would have a high half-life in the blubber), and therefore, the alkenones found in the blubber may have been ingested in different seasons. If this was the case, the $U^{k'}_{37}$ measured in the blubber layers in summer may be averaging the alkenones incorporated during the summer and the previous winter, which would decrease $U^{k'}_{37}$. Further research should calculate the half-lives of the alkenones to better understand the information integrated in this tissue.

This study showed that the measurement of $U^{k'}_{37}$ is feasible in fin whale tissues and can be used to approximate the sea surface temperature where these animals feed. However, several questions should be answered before this technique can be applied in more complex studies. For example, it should be tested whether the alkenones can be found in other species feeding on higher trophic levels than the fin whales and how the transit through different species (e.g., ingestion, digestion, absorption) affects the $U^{k'}_{37}$ ratio. It should also be confirmed whether the alkenones bioaccumulate, and their half-lives in marine mammal tissues should be estimated. Once this information is obtained, the alkenone analysis has great potential in a variety of studies, from identifying feeding habitats to detecting ontogenetic or historical habitat changes.

References

Ackman, R. G., C. A. Eaton, and P. M. Jangaard. 1965. Lipids of the fin whale (*Balaenoptera physalus*) from North Atlantic waters. I. Fatty acid composition of whole blubber and blubber sections. Can. J. Biochem. **43**: 1513–1520. doi:10.1139/o65-169

Aguilar, A. 1985. Biología y dinámica poblacional del rorcual común (*Balaenoptera physalus*) en las aguas atlánticas ibéricas. Univ. de Barcelona. ISBN: 9788469341414.

Aguilar, A., and A. Borrell. 1990. Patterns of lipid content and stratification in the blubber of fin whales (*Balaenoptera physalus*). J. Mammal. **71**: 544–554. doi:10.2307/1381793

Aguilar, A., and A. Borrell. 1991. Heterogeneous distribution of organochlorine contaminants in the blubber of baleen whales: Implications for sampling procedures. Mar. Environ. Res. **31**: 275–286. doi:10.1016/0141-1136(91)90017-3

Aguilar, A., and R. García-Vernet. 3. Academic Press; 2018. Fin whale, *Balaenoptera physalus*, p. 368–371. *In*. Würsig Bernd, Thewissen J.G.M., Kovacs Kit M., Encyclopedia of marine mammals.

Bayes, S. K., M. K. Hellerstein, M. Fitch, N. J. Mills, and S. C. Welter. 2014. You are what you eat: Fatty acid profiles as a method to track the habitat movement of an insect. Oecologia **175**: 1073–1080. doi:10.1007/s00442-014-2976-9

Bendle J. A. P., and A. Rosell-Melé. 2007. High-resolution alkenone sea surface temperature variability in the North Icelandic Shelf: Implications for Nordic Seas paleoclimatic development during the Holocene. **17**(1): 9–24. doi: 10.1177/0959683607073269.

Borrell, A., E. Gómez-Campos, and A. Aguilar. 2016. Influence of reproduction on stable isotope ratios: Nitrogen and carbon isotope discrimination between mothers, fetuses, and milk in the fin whale, a capital breeder. Physiol. Biochem. Zool. **89**: 41–50. doi:10.1086/684632

Brassell, S. C., R. G. Brereton, G. Eglinton, J. Grimalt, G. Liebezeit, I. T. Marlowe, U. Pflaumann, and M. Sarnthein. 1986a. Palaeoclimatic signals recognized by chemometric treatment of molecular stratigraphic data. Org. Geochem. **10**: 649–660. doi:10.1016/S0146-6380(86)80001-8

Brassell, S. C., G. Eglinton, I. T. Marlowe, U. Pflaumann, and M. Sarnthein. 1986b. Molecular stratigraphy: A new tool for climatic assessment. Nature **324**: 129–133. doi:10.1038/324148a0

Brassell, S. C., and others. 2004. Recognition of alkenones in a lower Aptian porcellanite from the west-central Pacific. Org. Geochem. **35**: 181–188. doi:10.1016/j.orggeochem.2003.09.003

Caissie, B. E., J. Brigham-Grette, K. T. Lawrence, T. D. Herbert, and M. S. Cook. 2010. Last Glacial Maximum to Holocene sea surface conditions at Umnak Plateau, Bering Sea, as inferred from diatom, alkenone, and stable isotope records. Paleoceanography **25**: 1–16. doi:10.1029/2008PA001671

Cardona, L., I. Á. de Quevedo, A. Borrell, and A. Aguilar. 2012. Massive consumption of gelatinous plankton by mediterranean apex predators. PLoS One **7**: e31329. doi:10.1371/journal.pone.0031329

Carone, E., M. A. Pardo, S. Atkinson, K. Mashburn, H. Pérez-Puig, L. Enríquez-Paredes, and D. Gendron. 2019. Sex steroid hormones and behavior reveal seasonal reproduction in a resident fin whale population. Conserv. Physiol. **7**: 1–13. doi:10.1093/conphys/coz059

Cates, K. A., S. Atkinson, C. M. Gabriele, A. A. Pack, J. M. Straley, and S. Yin. 2019. Testosterone trends within and across seasons in male humpback whales (*Megaptera novaeangliae*)from Hawaii and Alaska. Gen. Comp. Endocrinol. **279**: 164–173. doi:10.1016/j.ygcen.2019.03.013

Conte, M. H., M.-A. Sicre, C. Rühlemann, J. C. Weber, S. Schulte, D. Schulz-Bull, and T. Blanz. 2006. Global temperature calibration of the alkenone unsaturation index ($U^{K'}_{37}$) in surface waters and comparison with surface sediments. Geochem. Geophys. Geosyst. **7**(2):1–22. doi: 10.1029/2005GC001054

de Leeuw, J. W., F. W. V. D. Meer, W. I. C. Rijpstra, and P. A. Schenck. 1980. On the occurrence and structural identification of long chain unsaturated ketones and hydrocarbons in sediments. Phys. Chem. Earth **12**: 211–217. doi:10.1016/0079-1946(79)90105-8

Grice, K., C. M. K. Breteler, S. Schouten, V. Grossi, J. W. de Leeuw, and J. S. S. Damsté. 1998. Effects of zooplankton herbivory on biomarker proxy records. Paleoceanography **13**: 686–693. doi:10.1029/98PA01871

Hobson, K. A., R. T. Alisauskas, and R. G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. Condor **95**: 388–394. doi:10.2307/1369361

Iverson, S. J., and H. N. Koopman. 2018. Blubber, 3rd Edition. Academic Press.

Kellar, N. M., J. Keliher, M. L. Trego, K. N. Catelani, C. Hanns, J. C. C. George, and C. Rosa. 2014. Variation of bowhead whale progesterone concentrations across demographic groups and sample matrices. Endanger. Species Res. **22**: 61–72. doi:10.3354/esr00537

Kennedy, J. A., and S. C. Brassell. 1992. Molecular records of twentieth-century El Niño events in laminated sediments from the Santa Barbara basin. Nature **357**: 62–64. doi:10.1038/357062a0

Leider, A., K. U. Hinrichs, G. Mollenhauer, and G. J. M. Versteegh. 2010. Core-top calibration of the lipid-based U37K′ and TEX86 temperature proxies on the southern Italian shelf (SW Adriatic Sea, Gulf of Taranto). Earth Planet. Sci. Lett. **300**: 112–124. doi:10.1016/j.epsl.2010.09.042

Lockyer, C. H., L. C. McConnell, and T. D. Waters. 1984. The biochemical composition of fin whale blubber. Can. J. Zool. **62**: 2553–2562. doi:10.1139/z84-373

Lourenço, R. A., M. M. De Mahiques, I. Elazari, K. Coaracy, A. Rosell-melé, and M. C. Bícego. 2016. Organic biomarker records spanning the last 34,800 years from the southeastern Brazilian upper slope: Links between sea surface temperature, displacement of the Brazil Current, and marine productivity. Geo-Mar. Lett. **36**: 361–369. doi:10.1007/s00367-016-0453-7

Marlowe, I. T., J. C. Green, A. C. Neal, S. C. Brassell, G. Eglinton, and P. A. Course. 1984. Long chain (n-c37-c39) alkenones in the prymnesiophyceae. Distribution of alkenones and other lipids and their taxonomic significance. Br. Phycol. J. **19**: 203–216. doi:10.1080/00071618400650221

McCaffrey, M. A., J. W. Farrington, and D. J. Repeta. 1990. The organic geochemistry of Peru margin surface sediments: I. A comparison of the C37 alkenone and historical El Niño records. Geochim. Cosmochim. Acta **54**: 1671–1682. doi:10.1016/0016-7037(90)90399-6

McIntyre, T., I. J. Ansorge, H. Bornemann, J. Plötz, C. A. Tosh, and M. N. Bester. 2011. Elephant seal dive behaviour is influenced by ocean temperature: Implications for climate change impacts on an ocean predator. Mar. Ecol. Prog. Ser. **441**: 257–272. doi:10.3354/meps09383

Nieto-Moreno, V., F. Martínez-Ruiz, V. Willmott, J. García-Orellana, P. Masqué, and J. S. Sinninghe Damsté. 2013. Climate conditions in the westernmost Mediterranean over the last two millennia: An integrated biomarker approach. Org. Geochem. **55**: 1–10. doi:10.1016/j.orggeochem.2012.11.001

Owen, K., K. C. S. Jenner, M. N. M. Jenner, R. D. McCauley, and R. D. Andrews. 2018. Water temperature correlates with baleen whale foraging behaviour at multiple scales in the Antarctic. Mar. Freshw. Res. **70**: 19. doi:10.1071/MF17288

Prahl, F. G., L. A. Muehlhausen, and D. L. Zahnle. 1988. Further evaluation of long-chain alkenones as indicators of paleoceanographic conditions. Geochim. Cosmochim. Acta **52**: 2303–2310. doi:10.1016/0016-7037(88)90132-9

Rasmussen, K., and others. 2007. Southern Hemisphere humpback whales wintering off Central America: Insights from water temperature into the longest mammalian migration. Biol. Lett. **3**: 302–305. doi:10.1098/rsbl.2007.0067

Reynolds, R., and D. Stokes. 1981. Reynolds NCEP Level 4 Optimally Interpolated SST Weekly Version 2. Ver. 2. PO. DAAC, CA, USA. doi: 10.5067/REYN2-OIMOW

Rosell-Melé, A., E. Bard, K. Emeis, J. O. Grimalt, P. J. Müller, and R. R. Schneider. 2001. Precision of the current methods to measure the alkenone proxy $U_{37}^{K'}$ and absolute alkenone abundance in sediments: Results of an interlaboratory comparison study. Geochem. Geophys. Geosyst. **2**(7):1–28. doi: 10.1029/2000GC000141.

Rowland, S. J., and J. K. Volkman. 1982. Biogenic and pollutant aliphatic hydrocarbons in *Mytilus edulis* from the North Sea. Mar. Environ. Res. **7**(2): 117–130. doi: 10.1016/0141-1136(82)90037-X.

Sachs, J. P., and S. J. Lehman. 1999. Subtropical North Atlantic temperatures 60,000 to 30,000 years ago. Science **286**: 756–760. doi:10.1126/science.286.5440.756

Sánchez-Montes, M. L., E. L. McClymont, J. M. Lloyd, J. Müller, E. A. Cowan, and C. Zorzi. 2020. Late Pliocene Cordilleran Ice Sheet development with warm northeast Pacific Sea surface temperatures. Clim. Past **16**: 299–313. doi:10.5194/cp-16-299-2020

Volkman, J. K., G. Eglinton, E. D. S. Corner, and J. R. Sargent. 1980. Novel unsaturated straight-chain C37-C39 methyl and ethyl ketones in marine sediments and a coccolithophore *Emiliania huxleyi*. Phys. Chem. Earth **12**: 219–227. doi:10.1016/0079-1946(79)90106-X

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Conflict of Interest

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Alkenone $U^{k'}_{37}$ index differs between thermally separated populations of fin whales and krill

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Abstract: Despite habitat temperature being an important determinant of the distribution and range of species, its measurement is difficult in the case of mobile marine organisms. Chemical tracers as alkenones are an alternative tool for gauging marine habitat temperature. The unsaturation index of alkenones, the $U^{k'}_{37}$ index, has been used by palaeoceanographers to estimate past sea surface temperatures (SST) and it can also be measured in living organisms. Here we analyse alkenones in a predator species, the fin whale, and its prey, the northern krill, both sampled in two areas with different SST: Iceland and NW Spain. In NW Spain (but not in W Iceland) alkenone concentrations were higher in krill than in fin whale blubber suggesting that, although they are transferred through the food web, they may become biodiluted and do not bioaccumulate. Consistently with local SST, the $U^{k'}_{37}$ index was lower in the Icelandic samples of both species than in those from NW Spain, confirming the ability of this index to discriminate populations that are thermally separated. While krill appeared to replace alkenones in about 10 days, fin whales did that at a much longer time scale. Whales are highly migratory animals, which implies that the alkenones present in their blubber are a mix of those ingested locally and in recently visited regions. Consistently with this, the $U^{k'}_{37}$ index correlated with average local SST in krill but not in fin whales. We conclude that alkenone replacement rate should be considered when using this proxy to assess feeding habitat of a species.

Title: Alkenone $U^{k'}_{37}$ index differs between thermally separated populations of fin whales and krill

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Keywords: *Meganyctiphanes norvegica, Balaeonoptera physalus*, North Atlantic, Temperature

Abstract

Despite habitat temperature being an important determinant of the distribution and range of species, its measurement is difficult in the case of mobile marine organisms. Chemical tracers as alkenones are an alternative tool for gauging marine habitat temperature. The unsaturation index of alkenones, the $U^{k'}_{37}$ index, has been used by palaeoceanographers to estimate past sea surface temperatures (SST) and it can also be measured in living organisms. Here we analyse alkenones in a predator species, the fin whale, and its prey, the northern krill, both sampled in two areas with different SST: Iceland and NW Spain. In NW Spain (but not in W Iceland) alkenone concentrations were higher in krill than in fin whale blubber suggesting that, although they are transferred through the food web, they may become biodiluted and do not bioaccumulate. Consistently with local SST, the $U^{k'}_{37}$ index was lower in the Icelandic samples of both species than in those from NW Spain, confirming the ability of this index to discriminate populations that are thermally separated. While krill appeared to replace alkenones in about 10 days, fin whales did that at a much longer time scale. Whales are highly migratory animals, which implies that the alkenones present in their blubber are a mix of those ingested locally and in recently visited regions. Consistently with this, the $U^{k'}_{37}$ index correlated with average local SST in krill but not in fin whales. We conclude that alkenone replacement rate should be considered when using this proxy to assess feeding habitat of a species.

Introduction

Temperature is a critical factor in the distribution of any living being (Hamazaki, 2002). It has a direct impact on the fitness of a species, i.e. its capacity to reproduce,

feed, grow and survive (Bronson, 1985; Russell et al., 2002) and it also affects the community in which the species lives (Richardson, 2004). Both factors limit the habitats used by a species, and they ultimately constrain its distribution range. Thus, ambient temperature is a basic component that needs to be monitored to establish the ecological niche of a species. However, ambient temperature cannot not always be straightforward measured, especially in the marine environment. For example, in species that have separate feeding and reproductive grounds, or that live in a wide temperature range, some of the occupied areas may remain unknown and thus its temperature impossible to determine. In these cases, a chemical tracer may be a useful tool to obtain information from those habitats.

Alkenones are a group of molecules often used by oceanographers to estimate water temperature. These molecules are exclusively produced by a group of haptophyte organisms, mainly *Emiliana huxleyi* (Marlowe et al., 1984), and they are ubiquitous in most oceans (Volkman, 2000). Although the name alkenone includes many molecules of different length (Volkman et al., 1980a), the two most used are the $C_{37:2}$ and $C_{37:3}$. The proportion of $C_{37:2}$ over the combined $C_{37:2}$ and $C_{37:3}$ is known as the $U^{k'}_{37}$ index (Prahl et al., 1988) and it is strongly correlated with the temperature of the water where the synthesising organisms grow (Conte et al., 2006; Prahl and Wakeham, 1987). Alkenones are extremely stable and accumulate in marine sediments, which permits its use to study the water temperature of the past (e.g. Brassell et al., 2004; McCaffrey et al., 1990; Sánchez-Montes et al., 2020). Although some organisms do not absorb alkenones (Grice et al., 1998; Rowland and Volkman, 1982; Volkman et al., 1980b), these molecules can enter the trophic web and be found in krill (Rita et al., 2020) as well as in the tissues of predators, such as in the blubber of some cetaceans (Rita et al., 2021, 2020). Thus, the analysis of alkenones in marine animals can provide information on the water temperature where a species feeds. This information may be valuable to assist in the identification of the feeding grounds of some species, to gauge the effect of global warming on them, or to differentiate populations that feed in thermally separated areas.

The goal of this study was to compare the alkenone concentration and the $U^{k'}_{37}$ index at two trophic levels in two widely separated geographical zones with contrasting sea surface water temperatures (SST). To do so, we analysed krill (*Meganyctiphanes norvegica*) and blubber tissue from fin whales (*Balaenoptera physalus*) taken, both of them, off NW Spain and SW Iceland. It should be noted that the fin whales from these locations belong to two independent populations or stocks which do not intermingle (International Whaling Comission, 2009; Vighi et al., 2016). Our hypothesis was that the alkenones detected in the krill would mirror those of the water in the location since the transition time from the synthesisers to the krill may be relatively short. At the same time, we wanted to investigate whether the same relationship exists at higher trophic levels, especially in a highly mobile species as the fin whale and investigate its variation patterns and differences between populations.

Methods

Samples of the internal blubber layer (adjacent to the muscle) and stomach content (krill) were collected from fin whales caught during commercial whaling operations in northwest Spain in 1984 and 1985 and in Iceland in 1986 (Table 1). All samples were immediately frozen and stored at -20 °C until analysis. From these, five krill and five fin whale samples from NW Spain had been analysed and published previously by Rita et al (2020).

Table 1 Number of samples analysed for each location, year and species.

Location	Year	Fin whale	Krill
NW Spain	1984	18	0
	1985	15	12
Iceland	1986	15	12

Samples were analysed following Rita et al (2020). Briefly, approximately 5 g of each sample were freeze-dried for 48 h. Once dried, 1 g of sample was homogenized and introduced in test tubes. 50 µl of internal standard (2-pentatriacontanone; 20 $ng \cdot \mu l^{-1}$ in n-hexane) was added to the test tubes. Later, samples were saponified in methanolic KOH solution (4 ml H_2O:MeOH, 1:9; 3M KOH) for 60 min at 80 °C. The non-saponifiable lipids were extracted three times using n-hexane (4 ml). The extracted material was combined with KOH water solution (12 ml H_2O; 3M KOH) and the mix was vortexed and centrifuged; the n-hexane phase was separated and passed through Na_2SO_4 to eliminate any possible water remains. The n-hexane was reduced to 1-2 ml under the N2 stream and then purified using solid-phase extraction (Supelclean LC-NH2 SPE tubes; 3ml). Two fractions of increasing polarity (hydrocarbons and ketones) were obtained by elution with n-hexane (4 ml) and n-hexane:DCM 3:1 (v/v; 6ml), respectively. The second fraction was evaporated under a N_2 stream and dissolved in 50 µl of n-hexane before the gas chromatography (GC).

Chromatographic analysis was carried out on a Shimadzu GCMS-QP2010 equipped with a 30 m Sapiens-X5MS silica capillary column (0.25 mm ID, 0.25 µm fil thickness) and a mass spectrometer (MS) detector. Helium was the carrier gas with a flow of 1 $ml \cdot min^{-1}$. The GC temperature program was as follows: injection at 60 °C; 1 min isothermal 60 °C to 310 °C at 40 $°C \cdot min^{-1}$; and 20 min isothermal with a total run time of 36 min. Peak identification of C_{37} alkenones was based on the retention time of the peak and the comparison of the ion spectrum with those of pure alkenone standards. The concentration of each alkenone was quantified using the area of the ion with m/z 81.

$U^{k'}_{37}$ was calculated as:

$$U^{k\prime}_{37} = \frac{[C_{37:2}]}{[C_{37:2}]+[C_{37:3}]}$$

where $[C_{37:2}]$ and $[C_{37:3}]$ are the concentrations of each alkenone in the sample (Prahl and Wakeham, 1987). $U^{k'}_{37}$ was later transformed to temperature ($U^{k'}_{37}$-temperature) using the Conte et al. (2006) equation for the Atlantic region:

$$U^{k\prime}_{37}\text{–}\,temperature = 48.673(U^{k'}_{37})^3 - 94.569\left(U^{k'}_{37}\right)^2 + 80.716\left(U^{k'}_{37}\right) - 5.977$$

For each sample, the $U^{k'}_{37}$-temperature was compared to the actual measured temperature, which was obtained from the AVHRR_OI_NCEI-L4.GLOB.v2.0 dataset. This measured temperature was calculated as the average sea surface temperature present in each area. The coordinates of the feeding ground in NW Spain and Iceland were extracted from Sanpera and Aguilar (1992) and Víkingsson (1997), respectively. Since the transmission of alkenones from their synthesis to the animals is not instantaneous, there was a lag between the temperature measured in the environment and the $U^{k'}_{37}$-temperature measured in the tissues. To account for this lag, the correlation between the krill $U^{k'}_{37}$-temperature and the measured temperature was calculated applying a set of lags (from 0 days to -30 days). The highest correlation was found when using a lag of -10 days, and, therefore, all further analyses were made comparing the $U^{k'}_{37}$-temperature to the sea surface temperature 10 days before the capture of the whale.

The variability of the $U^{k'}_{37}$ index was extremely high when the concentration of the alkenones was low. This was probably due to the analytical error being more important when the concentration was low (Grimalt et al., 2001). For this reason, those samples with an alkenone concentration lower than 70 $ng \cdot g^{-1}$ were not considered for the $U^{k'}_{37}$ and $U^{k'}_{37}$-temperature analysis. This resulted in the exclusion of 10 fin whale samples from NW Spain, 5 krill samples from Iceland and 2 fin whale samples from Iceland. Also, 2 fin whale samples from NW Spain did not appeared to contain alkenones. The analyses were repeated with the same result.

Data analysis was performed in the statistical program R version 4.0.3 (R Core Team, 2020) with RStudio interface version 1.1.447 (RStudio Team, 2016). Homoscedasticity and normality of the data were tested using Bartlett's test and the Shapiro test, respectively. Alkenone concentration and $U^{k'}_{37}$ index data were not homoscedastic; thus, non-parametric tests (Kruskal-Wallis test) were used to detect significant differences between groups. Pairwise Wilcoxon Rank Sum tests were used as a *post hoc* analysis to detect differences between group pairs. The linear model was used to measure the correlation between the $U^{k'}_{37}$- temperature and the measured temperature. The residuals of the model were used to check the homoscedasticity of the data, and the QQ-plot was used to detect departures from normality. Linear models

were also used to examine the bioaccumulation of the alkenones in the blubber using the age of the individual as explanatory variable. Finally, the variation of the alkenone concentration over time was analysed with a general additive model (gam) using the date of capture as a smooth variable and the location and species as fixed variables.

Results

Alkenone concentration was significantly different among the interaction of area and species (χ^2 = 21.53; df = 3; p-value <0.001; Fig. 1). Specifically, in NW Spain, the alkenone concentration (mean ±SD) was significantly higher in the krill than in the fin whales (krill: 2485 ±2363 ng·g^{-1}; fin whale: 203 ±217 ng·g^{-1}; p-value <0.001), while in Iceland there was no significant difference between the two species (krill = 349 ±331 ng·g^{-1}; fin whale: 231 ±211 ng·g^{-1}; p-value = 1). Also, the alkenone concentration in fin whales was higher in Iceland than in NW Spain (p-value = 0.017), while the opposite was true for the krill samples (p-value = 0.015) (Fig. 1). No correlation was detected between the concentration of alkenones and the age of the whales (NW Spain: t value =-0.891, p-value = 0.39; Iceland: t-value = 0.18, p-value = 0.85). The gam analysis showed no effect between the date of sampling and the concentration of alkenones in neither the krill nor the whales from NW Spain (krill: F = 0.34, p-value = 0.574; fin whale: F = 1.07, p-value = 0.38) nor in the fin whales from Iceland (F = 1.55, p-value = 0.23), but there was an effect in the Icelandic krill (F = 5.255, p-value = 0.03).

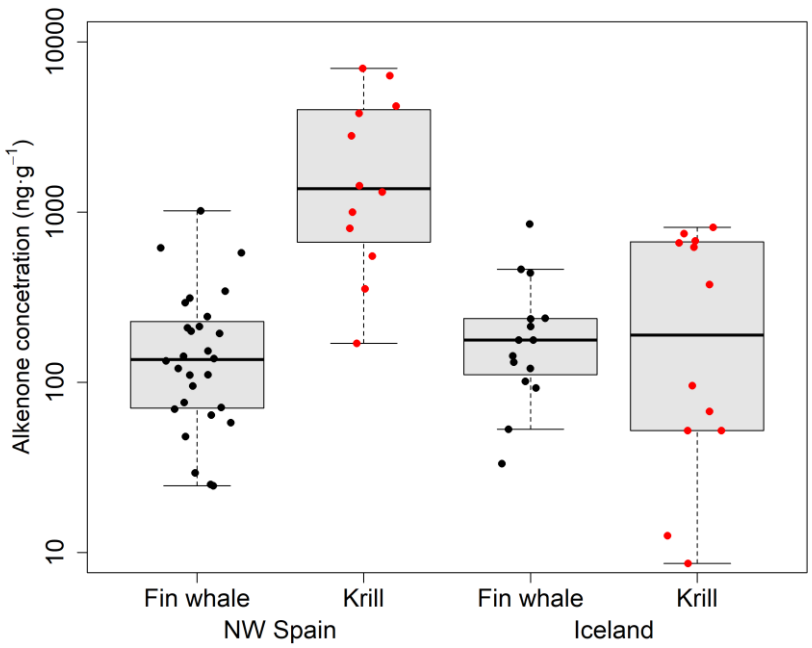

Figure 1. Alkenone concentration in krill and fin whales in the two locations. Note the log-transformed scale of the vertical axis.

The $U^{k'}_{37}$ index was significantly different between the two studied areas (F = 129; df = 1, 51; p-value <0.001; Fig. 2), but not between the fin whales and krill within each area (Iceland χ^2 = 0.26 df = 1, p-value = 0.6; Spain: F = 0.68; df = 1,31; p-value = 0.41). In NW Spain, the variability of the $U^{k'}_{37}$ index in fin whales was slightly higher than that of krill (fin whale: SD = 0.094; krill: SD = 0.076), but in Iceland the differences were more apparent (krill: SD = 0.017; fin whale: SD = 0.12).

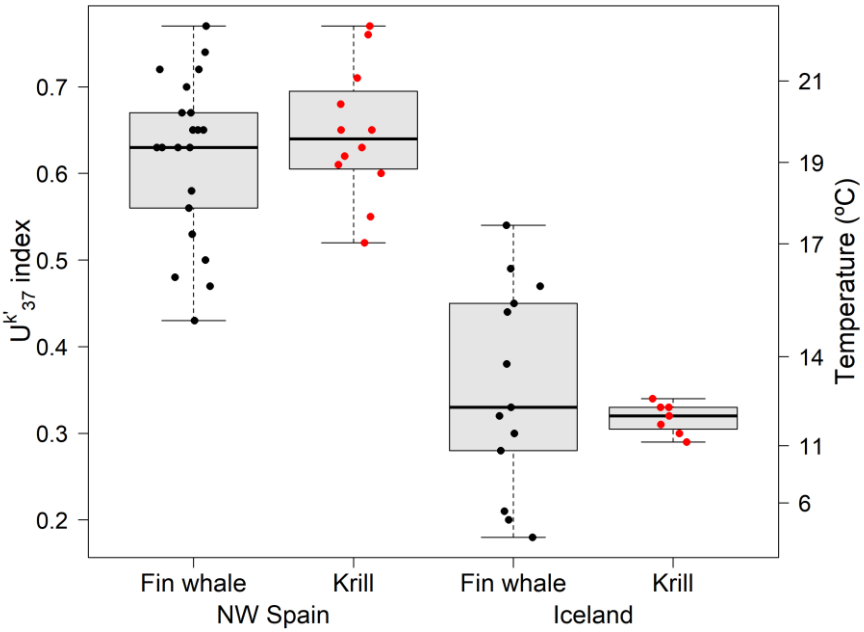

Figure 2. Mean, sdandard deviations and maximum and minimum values of the $U^{k'}_{37}$ index measured in krill and fin whale blubber in the two locations.

The sea surface temperature, measured 10 days before the capture of the whale, was significantly correlated with the estimated temperature in the krill from NW Spain (r^2 = 0.44; p-value = 0.017) but not in that from Iceland (r^2 = 0.1; p-value = 0.48) nor in any of the two fin whale populations (NW Spain: r^2 = 0.04, p-value = 0.38; Iceland: r^2 = 0.02, p-value = 0.57) (Figure 3).

Discussion

The results of this study show that alkenones can be detected in two different trophic levels, thus confirming previous research that indicated that they are transferred through the trophic web (Rita et al., 2020). The concentration of the alkenones was similar in krill and in fin whales in Iceland, but higher in the krill in NW Spain. This may indicate either that alkenones are biodiluted during transfer or that they maintain similar concentration through trophic transfer but, whatever the case, it shows that they are not biomagnified. Also, we found a lack of correlation between the alkenone

concentration and the age of the whales, a finding that indicates that these compounds do not bioaccumulate in the fin whale blubber. Although we cannot discard that alkenones may be present in other tissues in measurable concentrations, this is unlikely because their lipophilic nature would favour a preferential accumulation in the adipose tissue rather than in other, less lipid-rich tissues.

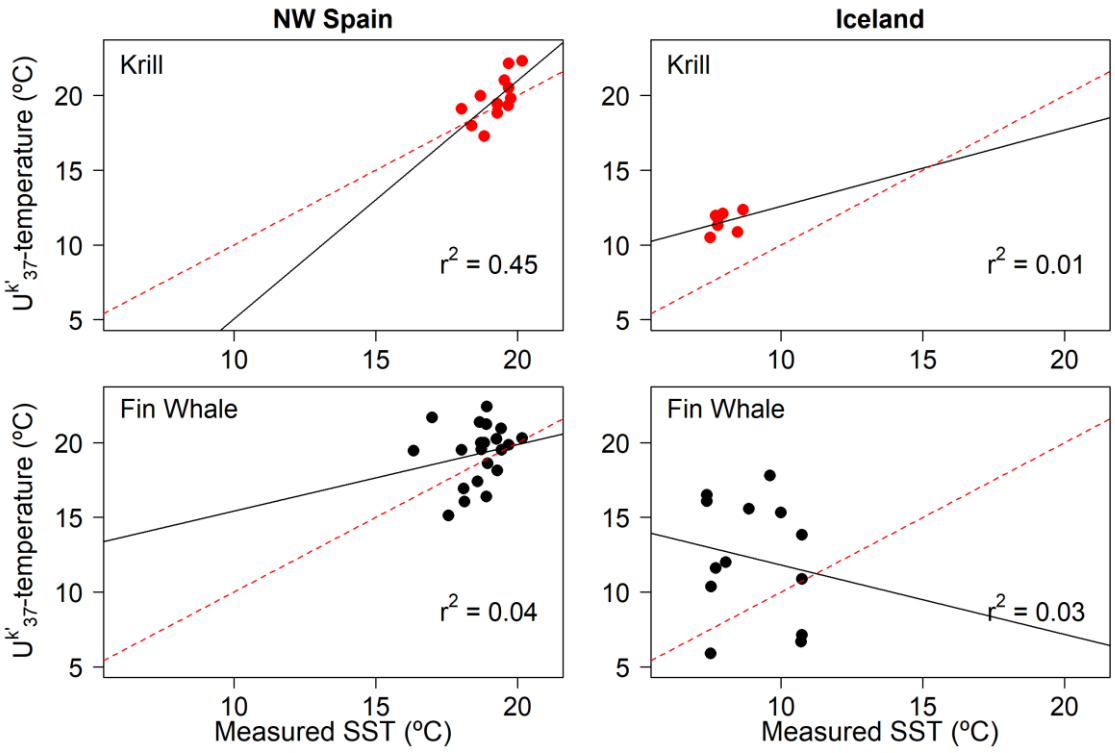

Figure 3. Correlation between the measured average sea surface temperature (SST) and the estimated temperature from the $U^{k'}_{37}$ index ($U^{k'}_{37}$-temperature) analysed in the krill and fin whale blubber from NW Spain and Iceland. The best correlation between the variable is shown as a solid black line. The red dashed line represents the 1:1 relation between measured and estimated temperature. The r^2 of the correlation is provided for each correlation in the lower right corner.

Alkenone concentration is strongly correlated with the abundance of haptophytes, which can bloom in the two studied locations (Conte and Eglinton, 1993). In suspended material, seasonal variations usually have a stronger effect on alkenone concentration than geographical variations (Conte et al., 2001; Conte and Eglinton, 1993; Prahl et al., 2005). In our study, the alkenone concentration in krill, which is expected to reflect the local alkenone production, showed significant differences between the two studied locations. Also, in Iceland the concentration was affected by sampling date, while no consistent temporal trend was present in the samples from NW Spain. These results seem to suggest that the alkenone concentration in krill is

influenced by short term variations of alkenone production. In fin whales, the lack of short- or long-term seasonal variation may be explained by the alkenone replacement rate in the tissues. Although alkenones do not bioaccumulate, their replacement in tissues is certainly not instantaneous. Thus, alkenones would remain in the tissue for some period of time and their composition would be an average of that period. This effect would tend to smooth any short-term variation in both the alkenone production and their ingestion by the whale.

The mean value of the $U^{k'}_{37}$ index was higher in NW Spain than in Iceland for both species, clearly as a response to the higher SST in NW Spain as compared to that in Iceland. These results are consistent with those obtained with another proxy for temperature previously investigated in the same fin whale populations, the oxygen stable isotope ratio or $\delta^{18}O$ value (Vighi et al., 2016), and confirm the ability of the $U^{k'}_{37}$ index to discriminate populations that are thermally separated. Also, because whales are highly mobile organisms and integrate in their tissues the heterogeneity of local environmental signals, the determination of this index in their blubber may be used, alone or in conjunction with other chemical proxies of temperature, to assess temperature shifts in large water masses (Borrell et al., 2018).

In Iceland, the variability of the $U^{k'}_{37}$ index measured in fin whales was much higher than that of krill while in NW Spain the difference in the variability between the two species was much smaller, largely due to a much higher variability of the index in krill. However, it should be noted that, although the variability in the $U^{k'}_{37}$ in the krill was similar to that of the fin whales in NW Spain, the variability in krill was largely explained by the seasonal change in water temperature, while the variability in fin whales was not. This is so because the alkenone replacement rate in fin whale tissues is very likely lower than in krill due to the larger body size of whales and therefore, their lower metabolic rate (White and Seymour, 2003). As a result, the alkenones detected in the fin whale blubber would be indeed a compendium of the food ingested not only locally during the stay of the individuals at the feeding grounds, but also of that consumed during the winter or along the autumn or spring migrations, which embrace water masses separated by thousands of kilometres. A further complication is that fin whales are capital breeders that accumulate large reserves of energy in the form of lipids to cope with the demands of reproduction and migration in a scenario where food availability is subject to strong seasonality (Lockyer, 1984). But blubber, the main body depot of lipids, is not a homogeneous tissue; rather, it is structured into layers and its composition varies between body regions reflecting heterogeneities in the dynamics of lipid deposition and mobilization both inside the tissue and between body locations (Aguilar and Borrell, 1990; Lockyer et al., 1984). Consequently, not all blubber depots are mobilized at the same speed or rate, and this would also contribute to introduce some variation within the sampling. To this, it should be added that, similarly as in other oceans (Mizroch et al., 2009; Shabangu et al., 2020; Soule and Wilcock, 2013), a small fraction of the fin whale populations may not engage in migration and thus overwinter in the feeding grounds, as the historical catch statistics show in Spain (Sanpera and

Aguilar, 1992) and the observations made from research vessels conducting winter capelin surveys evidence in Iceland (Gunnlaugsson and Víkingsson, 2014). All this combined is likely to explain the higher variability in the $U^{k'}_{37}$ index of fin whales as opposed to that of krill.

Consistently with the above, we found that the $U^{k'}_{37}$-temperature and the average SST measured 10 days before sampling were highly correlated in krill in NW Spain, but not in fin whales. This is again taken as an indication of a faster replacement of alkenones in krill than in whales. The lack of $U^{k'}_{37}$-temperature correlation in the Icelandic krill is probably due to the short period of time in which these latter samples were taken. The sampling period of the Icelandic krill elapsed a period of 21 days, which corresponds to a change in SST of 0.6 °C. This value is lower than the experimental error of alkenone quantification (Rita et al., 2020), and therefore the number of samples was inadequate to detect this change in temperature. It is probable that an increased number of samples, especially if they had been collected for longer period of time, would show similar trends in Iceland and in NW Spain. Finally, it is also probable that the small variability in the $U^{k'}_{37}$ index measured in Iceland is also a consequence of this short sampling period. A larger sampling over a longer period would probably increase the variance of the $U^{k'}_{37}$ index proportionally to the SST change over the season.

However, and surprisingly, the estimated temperature in Icelandic krill was 4.3 °C higher than expected. The reason for this discrepancy is unclear, but it does not appear to be related to the transport through the trophic web because previous studies in the region had found similarly high $U^{k'}_{37}$ indices in suspended organic matter (Conte and Eglinton, 1993; Rodrigo-Gámiz et al., 2015). A possible explanation for this may be that the alkenones present in the Icelandic krill had indeed been produced in warmer waters and transported horizontally (Benthien and Müller, 2000; Häggi et al., 2015). This hypothesis would be substantiated by the fact that Iceland is located at the northern end of the Gulf Stream extension region and is thus under the influence of a strong mid-latitude western current that brings water from warmer latitudes (Wu et al., 2020).

We can conclude that alkenones constitute a useful proxy of ambient temperature not only for primary producers but also for organisms situated at the top of the food web. They can differentiate thermally separated populations and contribute to determine the temperature range of the habitats they use. Thus, despite the complexity occasionally involved in the interpretation of the results, they provide an alternative insight into the biology of these organisms. In this context it is worth noting the apparent differences in the replacement rate of alkenones between organisms of different constitution. The high correlation between the estimated temperature in NW Spain krill and the sea surface temperature 10 days before sampling appears to suggest that this is the approximate replacement time in krill, a finding that may be relevant to future studies on krill habitat. The slightly higher variance in the $U^{k'}_{37}$ index in whales as compared to krill, and its lack of correlation with the local estimated sea surface temperature, indicate that the replacement rate in these animals is longer than in krill.

As a consequence, in large whales alkenones may only provide information over a protracted time scale.

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References

Aguilar, A., Borrell, A., 1990. Patterns of Lipid Content and Stratification in the Blubber of Fin Whales (Balaenoptera physalus). J. Mammal. 71, 544–554. https://doi.org/10.2307/1381793

Benthien, A., Müller, P.J., 2000. Anomalously low alkenone temperatures caused by lateral particle and sediment transport in the Malvinas Current region, western Argentine Basin. Deep. Res. Part I Oceanogr. Res. Pap. 47, 2369–2393. https://doi.org/10.1016/S0967-0637(00)00030-3

Borrell, A., Saiz, L., Víkingsson, G.A., Gaufier, P., López Fernández, A., Aguilar, A., 2018. Fin whales as bioindicators of multi-decadal change in carbon and oxygen stable isotope shifts in the North Atlantic. Mar. Environ. Res. 138, 129–134. https://doi.org/10.1016/j.marenvres.2018.04.014

Brassell, S.C., Dumitrescu, M., Bralower, T.J., Premoli-Silva, I., Malone, M.J., Arthur, M.A., Averyt, K., Bown, P., Channell, J.E.T., Clarke, L.J., Dutton, A.L., Eleson, J.W., Frank, T.D., Gylesjö, S., Hancock, H., Kano, H., Leckie, R.M., Marsaglia, K.M., McGuire, J., Moe, K.T., Petrizzo, M.R., Robinson, S., Röhl, U., Sager, W.W., Takeda, K., Thomas, D., Williams, T., Zachos, J.C., 2004. Recognition of alkenones in a lower Aptian porcellanite from the west-central Pacific. Org. Geochem. 35, 181–188. https://doi.org/10.1016/j.orggeochem.2003.09.003

Bronson, F.H., 1985. Mammalian Reproduction: An Ecological Perspective 1. Biol. Reprod. 32, 1–26. https://doi.org/10.1095/biolreprod32.1.1

Conte, M.H., Eglinton, G., 1993. Alkenone and alkenoate distributions within the euphotic zone of the eastern North Atlantic: correlation with production temperature. Deep. Res. Part I 40, 1935–1961. https://doi.org/10.1016/0967-0637(93)90040-A

Conte, M.H., Sicre, M.-A., Rühlemann, C., Weber, J.C., Schulte, S., Schulz-Bull, D., Blanz, T., 2006. Global temperature calibration of the alkenone unsaturation index ($U^{k'}_{37}$)

in surface waters and comparison with surface sediments. Geochemistry Geophys. Geosystems 7. https://doi.org/10.1029/2005GC001054

Conte, M.H., Weber, J.., King, L.., Wakeham, S.., 2001. The alkenone temperature signal in western North Atlantic surface waters. Geochim. Cosmochim. Acta 65, 4275–4287. https://doi.org/10.1016/S0016-7037(01)00718-9

Grice, K., Breteler, C.M.K., Schouten, S., Grossi, V., de Leeuw, J.W., Damsté, J.S.S., 1998. Effects of zooplankton herbivory on biomarker proxy records. Paleoceanography 13, 686–693. https://doi.org/http://dx.doi.org/10.1029/98PA01871

Grimalt, J.O., Calvo, E., Pelejero, C., 2001. Sea surface paleotemperature errors in UK'37 estimation due to alkenone measurements near the limit of detection. Paleoceanography 16, 226–232. https://doi.org/10.1029/1999PA000440

Gunnlaugsson, T.H., Víkingsson, G.A., 2014. Winter occurrence of whales in waters around Iceland., SC/65b/RMP06.

Häggi, C., Chiessi, C.M., Schefuß, E., 2015. Testing the D/H ratio of alkenones and palmitic acid as salinity proxies in the Amazon Plume. Biogeosciences 12, 7239–7249. https://doi.org/10.5194/bg-12-7239-2015

Hamazaki, T., 2002. Spatiotemporal prediction models of cetacean habitats in the mid-western North Atlantic Ocean (from Cape Hatteras, North Carolina, U.S.A. to Nova Scotia, Canada). Mar. Mammal Sci. 18, 920–939. https://doi.org/10.1111/j.1748-7692.2002.tb01082.x

International Whaling Comission, 2009. Report of the first intersessional RMP workshop on North Atlantic fin whales. J. Cetacean Res. Manag. 11, 425–452.

Lockyer, C., 1984. Review of baleen whale (Mysticeti) reproduction and implications for management. Reprod. whales, dolphins porpoises. Proc. Conf. La Jolla, CA, 1981 27–50.

Lockyer, C.H., McConnell, L.C., Waters, T.D., 1984. The biochemical composition of fin whale blubber. Can. J. Zool. 62, 2553–2562. https://doi.org/10.1139/z84-373

Lydersen, C., Garcia, J.V., Heide-Jørgensen, M.P., Øien, N., Guinet, C., Kovacs, K.M., 2020. Autumn movements of fin whales (*Balaenoptera physalus*) from Svalbard, Norway, revealed by satellite tracking. Sci. Rep. 10. https://doi.org/10.1038/s41598-020-73996-z

Marlowe, I.T., Green, J.C., Neal, A.C., Brassell, S.C., Eglinton, G., Course, P.A., 1984. Long chain (n-c37-c39) alkenones in the prymnesiophyceae. Distribution of alkenones and other lipids and their taxonomic significance. Br. Phycol. J. 19, 203–216. https://doi.org/10.1080/00071618400650221

McCaffrey, M.A., Farrington, J.W., Repeta, D.J., 1990. The organic geochemistry of Peru margin surface sediments: I. A comparison of the C37 alkenone and historical El Niño records. Geochim. Cosmochim. Acta 54, 1671–1682. https://doi.org/10.1016/0016-7037(90)90399-6

Mizroch, S.A., Rice, D.W., Zwiefelhofer, D., Waite, J., Perryman, W.L., 2009. Distribution

and movements of fin whales in the North Pacific Ocean. Mamm. Rev. 39, 193–227. https://doi.org/10.1111/j.1365-2907.2009.00147.x

Prahl, F.G., Muehlhausen, L.A., Zahnle, D.L., 1988. Further evaluation of long-chain alkenones as indicators of paleoceanographic conditions. Geochim. Cosmochim. Acta 52, 2303–2310. https://doi.org/10.1016/0016-7037(88)90132-9

Prahl, F.G., Popp, B.N., Karl, D.M., Sparrow, M.A., 2005. Ecology and biogeochemistry of alkenone production at Station ALOHA. Deep. Res. Part I Oceanogr. Res. Pap. 52, 699–719. https://doi.org/10.1016/j.dsr.2004.12.001

Prahl, F.G., Wakeham, S.G., 1987. Calibration of unsaturation patterns in long-chain ketone compositions for palaeotemperature assessment. Nature 330, 367–369. https://doi.org/10.1038/330367a0

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Richardson, A.J., 2004. Climate Impact on Plankton Ecosystems in the Northeast Atlantic. Science (80-.). 305, 1609–1612. https://doi.org/10.1126/science.1100958

Rita, D., Berdié, L., Borrell, A.A., Aguilar, A., 2020. Alkenones as a temperature proxy in fin whale (*Balaenoptera physalus*) tissues. Limnol. Oceanogr. Methods 18, 446–452. https://doi.org/10.1002/lom3.10375

Rita, D., Borrell, A., Aguilar, A., 2021. Alkenones in oceanic odontocetes as a potential proxy of environmental water temperature. Ecol. Indic. 122. https://doi.org/10.1016/j.ecolind.2020.107240

Rodrigo-Gámiz, M., Rampen, S.W., de Haas, H., Baas, M., Schouten, S., Sinninghe Damsté, J.S., 2015. Constraints on the applicability of the organic temperature proxies $U^{k''}_{37}$, TEX86 and LDI in the subpolar region around Iceland. Biogeosciences 6573–6590. https://doi.org/10.5194/bg-12-6573-2015

Rowland, S.., Volkman, J.., 1982. Biogenic and pollutant aliphatic hydrocarbons in Mytilus edulis from the North Sea. Mar. Environ. Res. 7, 117–130. https://doi.org/10.1016/0141-1136(82)90037-X

RStudio Team, 2016. RStudio: integrated development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Russell, A.F., Clutton-Brock, T.H., Brotherton, P.N.M., Sharpe, L.L., Mcilrath, G.M., Dalerum, F.D., Cameron, E.Z., Barnard, J.A., 2002. Factors affecting pup growth and survival in co-operatively breeding meerkats *Suricata suricatta*. J. Anim. Ecol. 71, 700–709. https://doi.org/10.1046/j.1365-2656.2002.00636.x

Sánchez-Montes, M.L., McClymont, E.L., Lloyd, J.M., Müller, J., Cowan, E.A., Zorzi, C., 2020. Late Pliocene Cordilleran Ice Sheet development with warm northeast Pacific sea surface temperatures. Clim. Past 16, 299–313. https://doi.org/10.5194/cp-16-299-2020

Sanpera, C., Aguilar, A., 1992. Modern whaling off the Iberian Peninsula during the 20th

century. Rep. - Int. Whal. Comm. 42, 723–730.

Shabangu, F., Andrew, R., Yemane, D., Findlay, K., 2020. Acoustic seasonality, behaviour and detection ranges of Antarctic blue and fin whales under different sea ice conditions off Antarctica. Endanger. Species Res. 43, 21–37. https://doi.org/10.3354/esr01050

Soule, D.C., Wilcock, W.S.D., 2013. Fin whale tracks recorded by a seismic network on the Juan de Fuca Ridge, Northeast Pacific Ocean. J. Acoust. Soc. Am. 133, 1751–1761. https://doi.org/10.1121/1.4774275

Vighi, M., Borrell, A., Aguilar, A., 2016. Stable isotope analysis and fin whale subpopulation structure in the eastern North Atlantic. Mar. Mammal Sci. 32, 535–551. https://doi.org/10.1111/mms.12283

Víkingsson, G.A., 1997. Feeding of fin whales (*Balaenoptera physalus*) off Iceland - diurnal and seasonal variation and possible rates. J. Northwest Atl. Fish. Sci. 22, 77–89. https://doi.org/10.2960/J.v22.a7

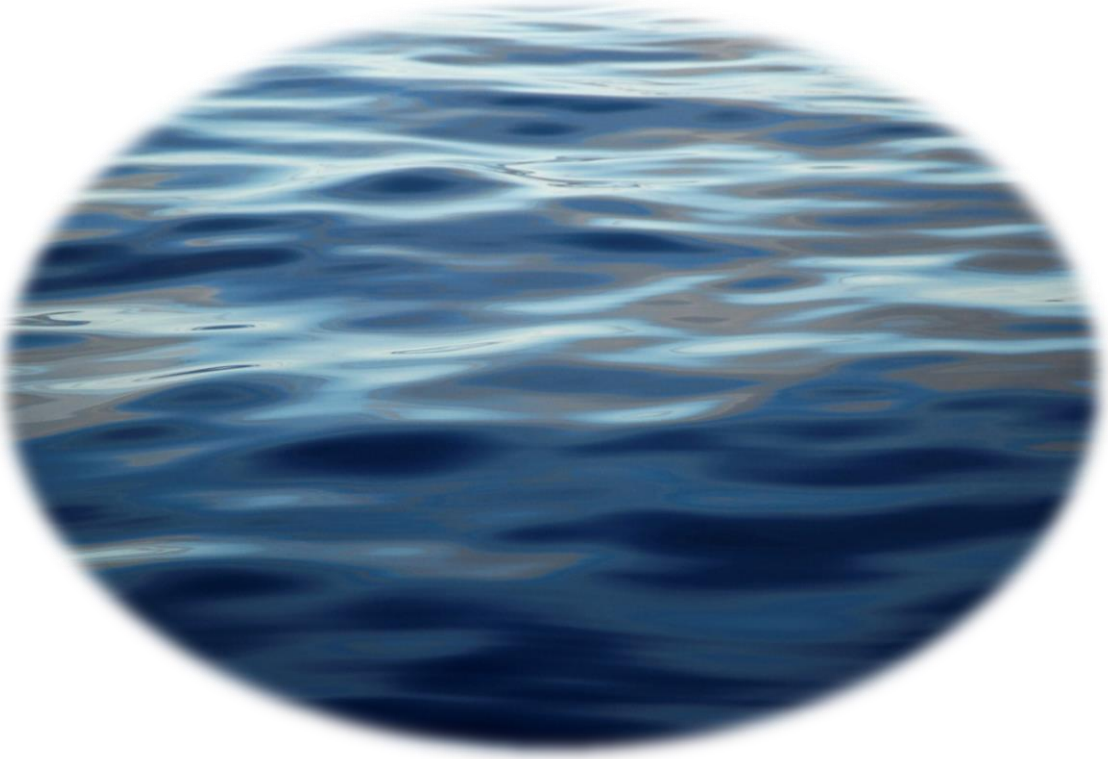
Volkman, J.K., 2000. Ecological and environmental factors affecting alkenone distributions in seawater and sediments. Geochemistry, Geophys. Geosystems 1. https://doi.org/10.1029/2000GC000061

Volkman, J.K., Eglinton, G., Corner, E.D.S., Forsberg, T.E. V, 1980a. Long-chain alkenes and alkenones in the marine coccolithophorid Emiliania huxleyi. Phytochemistry 19, 2619–2622. https://doi.org/10.1016/S0031-9422(00)83930-8

Volkman, J.K., Eglinton, G., Corner, E.D.S., Sargent, J.R., 1980b. Novel unsaturated straight-chain C37-C39 methyl and ethyl ketones in marine sediments and a coccolithophore Emiliania huxleyi. Phys. Chem. Earth 12, 219–227. https://doi.org/10.1016/0079-1946(79)90106-X

White, C.R., Seymour, R.S., 2003. Mammalian basal metabolic rate is proportional to body mass$^{2/3}$. Proc. Natl. Acad. Sci. U. S. A. 100, 4046–4049. https://doi.org/10.1073/pnas.0436428100

Wu, Y., Jia, Y., Ji, R., Zhang, J., 2020. SST warming in recent decades in the gulf stream extension region and its impact on atmospheric rivers. Atmosphere (Basel). 11. https://doi.org/10.3390/atmos11101109



Capítulo 2

Histological structure of baleen plates and its relevance to sampling for stable isotope studies

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Abstract: Stable isotope analysis of baleen plates is a widespread technique for studying baleen whales. Typically, subsamples along the growth axis of the baleen plate are extracted and analysed to examine time-related variation in their stable isotope signals. However, baleen plate tissue is composed of two different tissues: a pair of cortex layers flanking an internal medulla. These two histological components exhibit differential development, and their consolidation as a tissue is therefore likely non-synchronic. This could influence stable isotope results because the stable isotope signal may differ in each subsample according to the proportion of the two histological components extracted from the tissue. In this study, stable isotope analysis was combined with optical microscopy examination of fin whale (Balaenoptera physalus) baleen plates to understand the ontogeny of the two histological components. In both of them, the ^{15}N values followed a sinewave pattern along the growth axis of the baleen plate. However, the ^{15}N values of the cortex appeared to be advanced compared to those of the medulla. Additionally, the amplitude of the ^{15}N values in the oscillations was higher in the cortex than in the medulla. The histological examination revealed that these differences are caused by earlier and faster synthesis of the cortex layer compared to that of the medulla. Because the stable isotope ratios of the two layers differ, we propose that in this type of studies only the outer-most part (closest to the surface) of the cortex should be subsampled and analysed. Additionally, to include the most recently formed tissue, this subsampling should start well below the zwischensubstanz, or baleen "gum".

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Original investigation

Histological structure of baleen plates and its relevance to sampling for stable isotope studies

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ABSTRACT

Stable isotope analysis of baleen plates is a widespread technique for studying baleen whales. Typically, subsamples along the growth axis of the baleen plate are extracted and analysed to examine time-related variation in their stable isotope signals. However, baleen plate tissue is composed of two different tissues: a pair of cortex layers flanking an internal medulla. These two histological components exhibit differential development, and their consolidation as a tissue is therefore likely non-synchronic. This could influence stable isotope results because the stable isotope signal may differ in each subsample according to the proportion of the two histological components extracted from the tissue. In this study, stable isotope analysis was combined with optical microscopy examination of fin whale (*Balaenoptera physalus*) baleen plates to understand the ontogeny of the two histological components. In both of them, the $\delta^{15}N$ values followed a sinewave pattern along the growth axis of the baleen plate. However, the $\delta^{15}N$ values of the cortex appeared to be advanced compared to those of the medulla. Additionally, the amplitude of the $\delta^{15}N$ values in the oscillations was higher in the cortex than in the medulla. The histological examination revealed that these differences are caused by earlier and faster synthesis of the cortex layer compared to that of the medulla. Because the stable isotope ratios of the two layers differ, we propose that in this type of studies only the outer-most part (closest to the surface) of the cortex should be subsampled and analysed. Additionally, to include the most recently formed tissue, this subsampling should start well below the zwischensubstanz, or baleen "gum".

Introduction

Stable isotope ratios are widely used chemical tracers in animal ecology studies (Hobson, 1999; Peterson and Fry, 1987). These ratios provide information on a number of biological traits, such as the foraging ecology (Clementz and Koch, 2001), migration patterns (Bowen et al., 2005) and/or behavioural specialization of individuals (Bond et al., 2016; Rita et al., 2017). Metabolically inert tissues, such as whiskers, nails and hairs, are of particular relevance for this type of study. These tissues are formed by high-turnover stem cells that rapidly acquire the stable isotope ratios of the body pool (Ayliffe et al., 2004; Fry and Arnold, 1982). Once formed, their composition is fixed, and they do not exchange materials with the rest of the body, thus preserving a permanent and invariable record of the signal (Rubenstein and Hobson, 2004). Furthermore, tissues of this type that grow continuously store a time-series record of the stable isotope ratios of the body pool at various stages of the life cycle (Rubenstein and Hobson, 2004). In mysticetes, one such tissue that has proven particularly useful and has been the subject of numerous studies is the baleen plate (Busquets-Vass et al., 2017; DeHart and Picco, 2015; García-Vernet et al., 2018; Ryan et al., 2013).

The baleen plate is a keratinous tissue that grows on the upper jaw of all mysticetes (Rice, 2002). Its growth is continuous throughout the lifespan, and the longest plates of some individuals preserve information that covers over a decade in the case of balaenids (Hobson and Schell, 1998) or a few years in the case of balaenopterids (Aguilar et al., 2014). Typically, the stable isotopes of the baleen plates present annual oscillation due to prey switching, a change in baseline stable isotopes or fasting along the annual migratory route. However, there is no standardized method for baleen plate subsampling, and different studies may use different techniques. In some cases, baleen plate subsampling is performed by scraping off a thin layer of the baleen plate surface; in others, a

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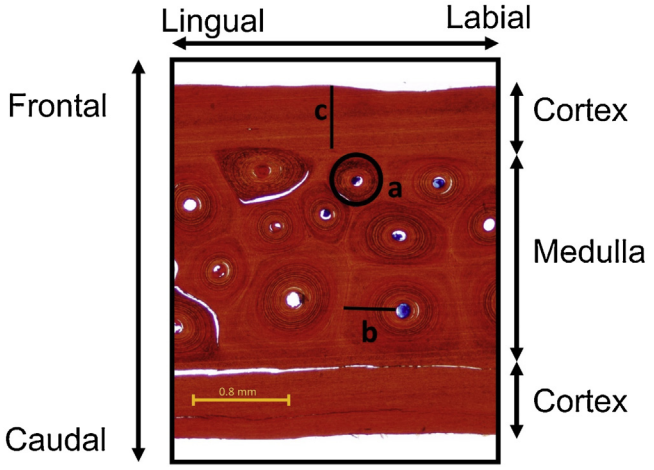

Fig. 1. Histological section of a fin whale baleen plate cut perpendicular to the growth axis of the plate (see orientation in Fig. 2). The image shows the three layers that constitute the plate: the two cortex layers and the medulla layer. The medulla layer is composed of horny tubules (a) that run parallel to the growth axis. The image also shows the two measurements used in this study: the width of the tubule wall (b) and the width of the cortex (c). A colour version of this figure can be found in the online version.

hole is drilled through the plate (Bentaleb et al., 2011; Busquets-Vass et al., 2017; Hunt et al., 2016; Mitani et al., 2006), and many studies do not specify the technique used (Eisenmann et al., 2016; Giménez et al., 2013; Hobson et al., 2004; Matthews and Ferguson, 2015). Different subsampling methods are likely to collect different components of the baleen plate since the baleen plate is not a homogenous tissue, potentially impacting the reliability and comparability of the results.

Baleen plates are composed of two histological components with a sandwich-type arrangement (Fig. 1). The medulla (the interior layer) consists of horny tubules that run parallel to the growing axis of the plate. The walls of these horny tubules are composed of concentric rings of flat and keratinized cells (Fig. 1) and, in some cases, calcium (Pfeiffer, 1992). The interior of the tubules, on the other hand, is filled with connective tissue near the base of the plate, but it may be empty in a mature plate (Pfeiffer, 1992). The medulla is flanked by two sheets of cortex (Fig. 1) composed of compact keratin filaments and flat cells (Fudge et al., 2009). During the early growth stages of the baleen plate, the medulla and the cortex sheets are separated by a third layer (Van Utrecht, 1966), which could play a role in the development of the other two layers. All histological components are synthesised inside the baleen "gum", or zwischensubstanz (Pinto and Shadwick, 2013; Van Utrecht, 1966; Young et al., 2015) (a white rubbery tissue that supports the base of the baleen plates). However, the exact depth at which the synthesis of each component occurs, where the tissue becomes completely keratinized, ("origin point" hereafter) is unknown. Previous histological studies have shown that the synthesis of the cortex occurs before that of the medulla (Van Utrecht, 1966), and this lack of synchronicity in tissue formation may cause a lag between the stable isotope ratios of the two components at any given tissue location.

The baleen plates used in this study belonged to two fin whales (*Balaenoptera physalus*) from the Icelandic population. This population feeds near Iceland during the boreal summer and migrates to lower latitudes of the North Atlantic during winter, a shift that is known to imprint an oscillatory pattern on the stable isotope ratios along the baleen plate growth axis (Aguilar et al., 2014; García-Vernet et al., 2018). The baleen plates of fin whales may include up to three of these oscillations since they grow at a rate of 18–23 cm (mean = 20 cm·year^{-1}) and measure up to 60 cm in length (Aguilar et al., 2014). These oscillations can be used to cross-correlate the isotopic signals of the two baleen plate layers to detect any time lag between the two layers.

The aim of the present study was to examine the process of the synthesis of the baleen plate components and the consequences that this process may have on the subsampling of the tissue. This new information may help to improve the accuracy and replicability of studies on stable isotope ratios in baleen plates. To this end, we performed histological examinations and stable isotope analysis at subsampling points along the growth axis of the baleen plates.

Material and methods

Sample collection

The baleen plates analysed in this study were obtained from two fin whales that were captured during commercial whaling operations in Iceland in 2015: whale #81, a juvenile 16.5-metre male caught at 64°25Ń-28°02′W; and whale #97, a 19.2-metre female pregnant with a 134-centimetre foetus, caught at 64°00Ń-27°48′W. One baleen plate collected from the middle section of the right jaw was excised from each animal at the very proximal end, in contact with the jawbone, to ensure that the whole plate had been sampled. The plate of whale #97 measured 67 cm, and the plate of whale #81 measured 63 cm. The plates were kept frozen at -20 °C until analysis.

Histological sections

From each baleen plate, we excised two stripes running parallel to the growing axis of the plate, one along and near the labial margin and the other along and near the lingual margin. The width of each stripe was 1 cm. Then, each of them was cut at distances of 1 cm, thus obtaining 1 cm x 1 cm square samples (Fig. 2). After concluding the process of synthesis, the histological structure of the plate remains essentially unaltered with the passing of time. Therefore, we focused our study on the synthesis portion of the baleen plate, which is the one located mostly underneath the baleen "gum" (zwischensubstanz) and, consequently, the histological examination of this portion was made in greater detail. Thus, the sampling consisted of one sample every centimetre in the region of the baleen plate inside the "gum" and one every 10 cm in the rest of the baleen plate (Fig. 2). Each sample, which had been kept frozen during the cutting process, was subsequently immersed for 24 h in 4% formaldehyde to preserve the histological structure; 4% formaldehyde and 30% sucrose to maintain the preservation process and initiate cryoprotection; 40 mM phosphate-buffered saline and 30% sucrose to extract the excess formaldehyde from the samples; and cryoprotectant solution (30% glycerol, 30% ethylene glycol in phosphate buffer 0.1 M) to further protect the histological samples from the cold temperatures of the cryostat. Then, the samples were frozen using dry ice, and histological sections were obtained using a cryostat. From each sample, several 20 μm-thick histological sections were cut perpendicular to the growing axis of the plate.

The sections from the samples of the labial strip of the plate from whale #81 were stained using haematoxylin-eosin. However, better results were obtained using Mallory's trichrome technique; therefore, the remaining samples (i.e., the lingual strip of the plate from whale #81 and both strips of the plate from whale #97) were stained using that technique. These two staining techniques were chosen because they are standard methods that have consistently proven to be useful for observation of the baleen plate tissue (i.e. Van Utrecht, 1966).The first staining technique only discriminated the basic cell components from the acidic cell components, such

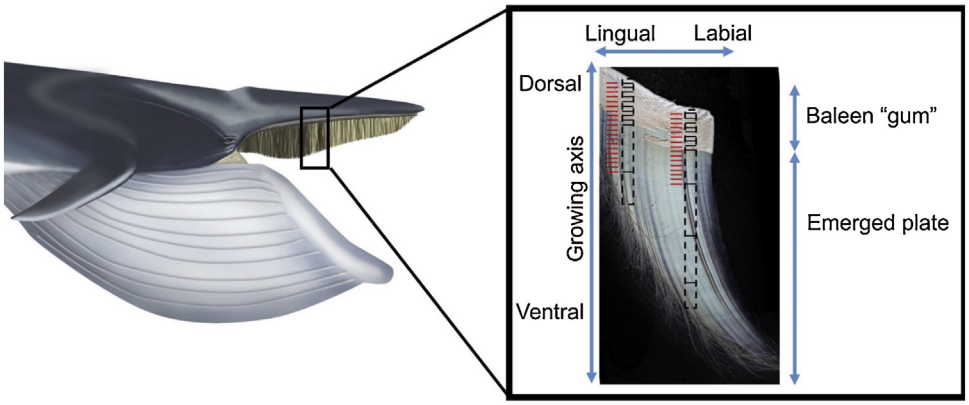

Fig. 2. Position of the baleen plates in vivo (left) and sampling scheme of the baleen plate (right). The white area in the dorsal zone of the plate is the baleen plate "gum" or zwischensubstanz. The dashed rectangles represent the 1 cm strips cut with the circular saw (not to scale). The red lines represent the stable isotope sampling points, and the black lines show the orientation of the samples used to obtain the histological sections. A colour version of this figure can be found in the online version.

as the nucleus from the cytoplasm, while the Mallory technique stained collagen, keratin and cytoplasm with different colours.

During the histological examination, we observed that the cortex and the horny tubules of the medulla were extremely thin at the base of the plate and became thicker at more distal positions until they reached their maximum thickness in the fully developed plate. Thus, we decided to use the width of the cortex and the width of the medullar tubules as proxies of cortex development and medulla development, respectively (Fig. 1). Cortex width, tubule diameter and tubule lumen diameter were measured in each subsample using an ocular micrometre under the optic microscope. Tubule wall width was calculated as the difference between the tubule radius and the tubule lumen radius. Each measurement was repeated in different sections from each sample (a minimum of seven measurements were conducted for each sample, but 20 measurements were performed when possible). The results reported here are the mean of all the measurements of one subsample. For further details, including the standard deviations obtained along the measurements, see Supplementary Data SD1.

Stable isotope analysis

Nitrogen and carbon stable isotope ratios were analysed from the lingual and labial sides of the baleen plates. Before drilling the baleen plate subsamples, the "gum" was removed using a scalpel, and the plate surface was rinsed using chloroform/methanol (2:1) solution. This removed possible surface lipids that might impact the measured isotopic values. Subsamples were collected from the zone inside the "gum" at adjacent locations to those subjected to histological examination and from the zone outside the "gum" every 1 cm along the first 10 cm of the emerged plate (Fig. 2). A Dremel 300 series drill was used to obtain powder from the cortex of the plate by carefully scratching its surface. For the medulla samples, the cortex was removed using the drill until the medulla tubules could be seen, and after rinsing with 96% ethanol, the medulla was scratched using the drill. To ensure that only medulla was sampled, the path carved in the cortex was wide enough to guarantee that the drill tip would not touch the cortex walls of the path while sampling the medulla.

Approximately 0.3 mg of baleen keratin was weighed into tin cups (3.3 x 5 mm) and analysed along with calibration standards by elemental analysis isotope ratio mass spectrometry (EA-IRMS) using an elemental analyser (model FlashEA 1112, ThermoFisher Scientific) coupled with a Delta C isotope ratio mass spectrometer (ThermoFinnigan). All analyses were performed at the Scientific and Technological Centre (CCiT) of the University of Barcelona.

Nitrogen stable isotope abundances are expressed in delta (δ) notation, with relative variations of stable isotope ratios expressed in per mil (‰) deviations from predefined international standards (Bond and Hobson, 2012). The international standards used were atmospheric nitrogen (air) for $\delta^{15}N$ and Vienna Pee Dee Belemnite (VPDB) calcium carbonate for $\delta^{13}C$. However, the data were normalized using commercially available laboratory reference materials. Secondary isotopic reference materials of known ^{15}N:^{14}N ratios, as given by the International Atomic Energy Agency (IAEA, Vienna, Austria), were used for calibration at a precision of 0.05%. These materials included $(NH_4)_2SO_4$ (IAEA-N-1, $\delta^{15}N$ = +0.4 ‰ and IAEA-*N*-2, $\delta^{15}N$ = +20.3 ‰), L-glutamic acid (IAEA USGS-40, $\delta^{15}N$= -4.5 ‰) and KNO_3 (IAEA-N-3, $\delta^{15}N$= +4.7 ‰). For carbon, the secondary isotopic reference materials with known ^{13}C:^{12}C ratios included polyethylene (IAEA-CH-7, $\delta^{13}C$ = -32.1‰), L-glutamic acid (IAEA USGS-40, $\delta^{13}C$ = -26.4 ‰) and sucrose (IAEA-CH-6, $\delta^{13}C$ = -10.4 ‰). These isotopic reference materials were assessed once per 12 analysed samples to recalibrate the system and compensate for any measurement drift over time. The raw data were normalized by the multipoint normalization method based on linear regression (Skrzypek, 2013).

The results regarding carbon stable isotope values lacked the standard and well-defined oscillation pattern that is necessary to carry out cross-correlation analysis. For this reason, only the nitrogen stable isotope values were used for the statistical analysis. For further details on the stable isotope values of carbon and nitrogen, see Supplementary Data SD2.

Statistical analysis

All statistical analyses were carried out using the free software R (R Core Team, 2016). The lag between the stable isotope ratios of the baleen cortex and the baleen medulla was statistically calculated via cross-correlation. The peak-to-valley amplitude (hereafter referred to as the amplitude) was calculated as the difference between the maximum and minimum $\delta^{15}N$ value of an oscillation.

The origin point of the cortex was estimated for each strip by fitting a linear regression between the cortex width values and the distance to the base of the section. The origin point was defined as the intersection of this linear regression with the x axis. Only measurements obtained before the point where the medulla began to exhibit keratinization were used for the linear regression.

A von Bertalanffy growth curve was fitted between the tubule wall width values and the distance to the plate base to estimate the origin point. The medulla keratinization period was calculated as

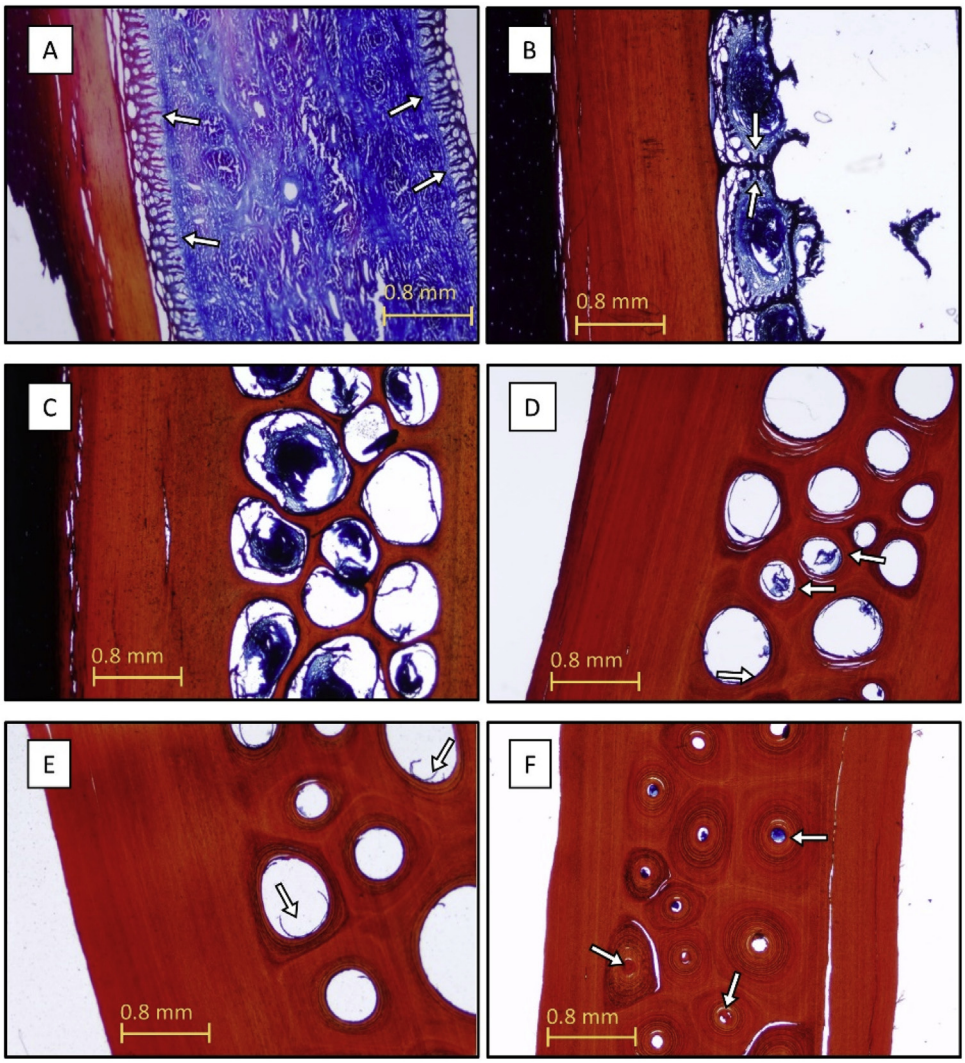

Fig. 3. Selection of photomicrographs of a baleen plate at different development stages. A shows the three-layer structure of the incipient plate: soft connective medulla (light blue), tissue between the cortex and the medulla (arrows) and cortex (orange); it also shows the "gum" (deep blue). B shows the segmented connective medulla with the evaginations facing the inside of the segments (arrows). C shows an incipient tubule with keratinized walls and a mature cortex. D shows the calcium rings and pigmentation granules around the tubules (arrows). E shows the cellular debris (arrows) inside the tubule lumen after the baleen plate has emerged from the "gum". F shows the cellular debris (arrows) inside the tubule lumen in the mature baleen plate. A colour version of this figure can be found in the online version.

the time required for the tubules to achieve 95% of their asymptotic width.

Results

At the most proximal position of the baleen plate, the histological sections showed that the cortex had already started to undergo keratinization, while the medulla was only composed of soft connective tissue (Fig. 3a). In this region, the two components were separated by a third layer (Fig. 3a; arrows) composed of two types of cells: flat keratinized cells near the cortex and round keratin-free cells near the medulla. Although the third layer formed a flat edge at its border with the cortex, at the border with the medulla, it formed evaginations that penetrated the medulla (Fig. 3a; arrows). The three-layer structure occurred approximately along the first 7.3 ± 3 cm of the plate, which was well within the "gum" region. Along this segment, the cortex progressively increased in thickness linearly when advancing distally (Fig. 4). When this segment ended, approximately 3-8 cm before the baleen emerged from the "gum", the structure abruptly changed. The layer between the cortex and the medulla formed filaments that crossed the medulla, dividing it into small sections (Fig. 3b). These sections eventually became the walls of the tubules in the mature medulla (Fig. 3c). In addition, the growth rate of the cortex was abruptly reduced by approximately 3 cm, after which its width stabilized (Fig. 4).

As the baleen plate grew, the tubule walls became thicker. Some concentric dark rings appeared around the lumen of the tubules (Fig. 3d; arrows) which had been previously identified as calcium rings by Pfeiffer (1992). The enlargement and keratinization of the tubule walls extended for approximately 20.65 ± 7.04 cm (Table 1) and ended well after the baleen plate had emerged from the "gum". Finally, during this stage, some pigment granules appeared in the medulla and became more frequent in the older parts of the plate (Fig. 3d; arrows).

The "gum" was approximately 12 cm thick. Once the baleen plate emerged from it, the cortex started to become eroded (Fig. 4). The connective tissue inside the tubules, which was always present under the "gum", remained in the first emerged centimetres but eventually disappeared. However, its disappearance may have been enhanced by the sectioning process given that some left-over tissue is sometimes present at positions situated more distally (Fig. 3e; arrows). This may indicate that the connective tissue is more

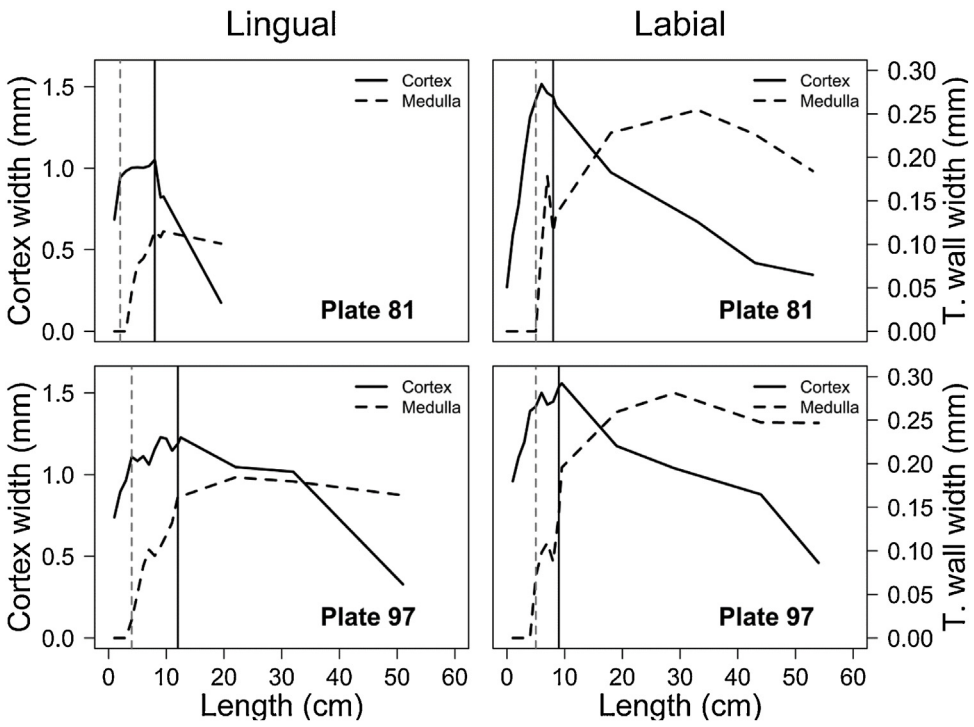

Fig. 4. Cortex and tubule wall width along the four baleen plate strips examined. The strips were extracted from the lingual (left) and labial (right) sides of the plates collected from whale #81 (top) and whale #97 (bottom). The dashed grey vertical line indicates the position at which the medulla starts to form. The continuous black vertical line indicates the position at which the plate emerges from the "gum". Length was measured from the dorsal-most part of the plate (i.e., the point closest to the bone).

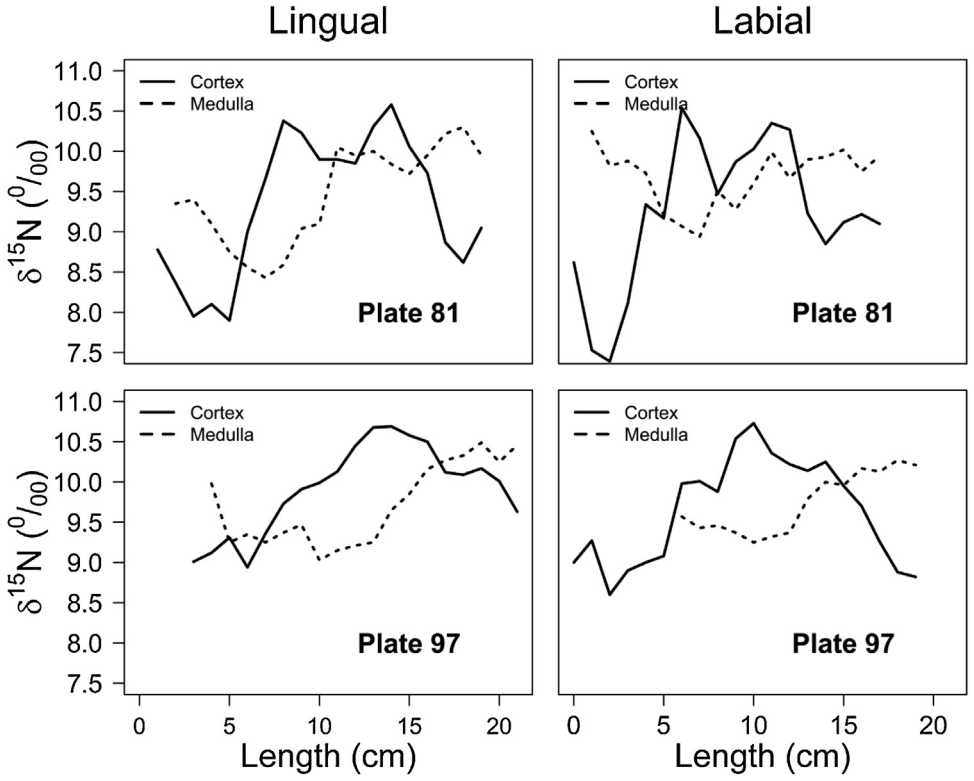

Fig. 5. Variation in the $\delta^{15}N$ values of the cortex and medulla along the four strips of baleen plates analysed. These strips correspond to the lingual (left) and labial (right) sides of plates from whales #81 (top) and #97 (bottom). Length was measured from the dorsal-most part of the plate (i.e., the point closest to the bone).

Table 1
Summary of the results for stable isotope ratios and histological measurements from the four baleen plates strips analysed.

		δ^{15}N value amplitude (‰)		δ^{15}N value lag (cm)	Growth period (cm)	
Whale	Section	Cortex	Medulla	Cortex-medulla	Cortex	Medulla
81	Labial	3.15	1.31	5	6.1	25.5
81	Lingual	2.68	1.87	3	3.7	11.1
97	Labial	2.13	1.02	7	10.4	26.4
97	Lingual	1.75	1.46	5	9.2	19.6
Mean		2.43 ± 0.61	1.42 ± 0.35	5 ± 1.6	7.4 ± 3.0	20.7 ± 7.0

loosely attached to the tubule walls in the more distal positions. In the fully formed tubules, the lumen appeared empty or filled with either a vacuole (or possibly cellular debris (Szewciw et al., 2010)) or keratin (Fig. 3f; arrows).

The δ^{15}N values followed one sinusoid oscillation along the 20 cm of sampled baleen plate in both the cortex and the medulla (Fig. 5). However, the amplitude of the variation of the δ^{15}N values was approximately 78 % higher in the cortex than in the medulla (Table 1). The cross-correlation of the two δ^{15}N values showed that the medulla values were 5 ± 1.6 cm lagged (equivalent to three months according to a growth rate of 20 cm·year^{-1}) related to the corresponding values in the cortex (Table 1).

Discussion

The histological examination confirmed that the baleen plate was keratinized from the outside in (Pinto and Shadwick, 2013). It also showed that the cortex formed first and that its origin point was at the very base of the baleen plate. Once the baleen plate emerged from the "gum" the width of the cortex decreased, possibly due to erosion (Werth et al., 2016). While the synthesis of the cortex was linear and fast, the synthesis of the medulla was slow and the increase in tubule thickness fit a von Bertalanffy growth curve.

The present results are relevant to studies involving stable isotope analysis because the dissimilar processes of the formation of the cortex and the medulla are likely to result in different stable isotope values depending on which component of the baleen plate is subsampled. In other words, the shorter growth period of the cortex compared to that of the medulla reduces the period during which the cortex integrates stable isotopic information. This likely increases the resolution of the cortex time-series record, which in turn increases the amplitude of the δ^{15}N oscillations. On the other hand, the long period required for the medulla integration would act as a moving average point, which would dampen the variability of the isotopic signal. In addition, the difference in location along the baleen plate of the origin point of the two components produces a lag, equivalent to a 3-month lag, between the δ^{15}N values of the medulla with respect to those of the cortex. We expected this lag to be the same as the distance existing between the respective origin points of each component, but we found in all cases that it was slightly shorter than that distance. The reason for this finding was unclear, but it may have been an artefact associated with the model fitted to calculate the growth period of the cortex. We assumed that the cortex grew following a linear model because the scant number of points that we had available showed this tendency. However, we suspect that, for a greater number of points/samples, a von Bertalanffy model would be more adequate and would likely place the origin point of the cortex closer to that of the medulla.

Although baleen plates have a clear curvature in their growth axis (Fig. 2), the cuts performed with the circular saw were all straight cuts. This is likely to produce an error in the measurements that would become larger in the ventral samples, where the curvature becomes more pronounced (Fig. 2). This error would cause a lower measured distance between the subsample and the base of the plate and would distort the histological examination since the sections would no longer be perfectly perpendicular to the growth axis. However, it should be noted that none of these errors would greatly impact our study because most of the measurements and the conclusions have been extracted from the synthesis zone of the plate, where the curvature is minimal.

These findings emphasize the need for standardization of the subsampling methods used in baleen plate stable isotope studies. Clearly, samples should be taken only from one layer, either the cortex or the medulla, and should never be collected as a combination of the two components. This is quite easy to achieve since the two histological components can be distinguished visually during the subsampling. After considering the two options, we recommend that the cortex should preferentially be analysed rather than the medulla for two reasons: a) it is close to the surface and is therefore easier to sample, and b) it integrates a shorter period, likely making its resolution in recording the stable isotope ratios higher. The increase in resolution not only provides a sharper picture of the migratory pattern, but also more accurate stable isotope values, something which is especially important when estimating diet or feeding grounds based on the stable isotope ratios. For example, an error in 1 ‰ in the δ^{15}N values would mask any potential difference between segments of the baleen plate deposited for example in Greenland and NW Spain (McMahon et al., 2013). Additionally, we strongly recommend starting the subsampling as far in the dorsal direction as possible and including the zone located underneath the "gum" because this is where the most recent stable isotope values (i.e., the origin point) are located.

However, this method involves a caveat related to the erosion suffered by the cortex layer once it emerges from the "gum" (Werth et al., 2016). As the cortex erodes, deeper layers of the cortex become exposed to the surface, and at the very tip of the baleen plate, only the cortex layer closest to the medulla will remain. Since the cortex closest to the medulla is synthesised after the cortex closest to the surface, it is expected that there will be a small lag between the respective isotope ratio values. The overall effect of this caveat would be a slight expansion of all the stable isotope oscillations in the baleen plate, which may somewhat skew the measurements of the growth rate. It is not clear how this error may be distributed along the baleen since different parts of the baleen may suffer different amounts of erosion. It is suspected that the zones with high erosion rates may also exhibit greater expansion of stable isotope oscillation.

Although this study was based on the results from only two baleen plates from two different individuals, we believe that its results are representative of the species because the synthesis of a tissue is a highly conservative process and tends to follow the same pattern within species. On the same grounds, we believe that the process is not affected by the age, sex or any other biological trait of the individual. Also, given that the baleen plate is a fully keratinized tissue, and that keratin does not experience changes or decomposition if properly stored, we consider that the conclusions apply to specimens properly stored in museum collections, irrespectively of their age.

Furthermore, the fact that the overall structure of the baleen plate is similar in blue (Fudge et al., 2009; Van Utrecht, 1966), minke, humpback, sei (Szewciw et al., 2010) and fin whales (Pinto and Shadwick, 2013; Van Utrecht, 1966) supports the idea that the above findings and recommendations are also applicable to other balaenopterid species. However, before the present results are extended to other species, it is recommended that confirmation studies be conducted because some small differences in the structure of the plate, particularly in the pattern of calcification or in the distribution of the tubules, are known to occur between species (Szewciw et al., 2010). Conversely, the plate of balaenids is much thicker and longer, and its pattern of formation may therefore not be directly comparable. Moreover, the above considerations may likely apply to other elements and molecules, such as heavy metals (Hobson et al., 2004) and hormones (Hunt et al., 2016), whose analyses can also be carried out in baleen plates. The only exception to this situation would be for elements that are progressively adsorbed into the plate from the surrounding seawater, as occurs for strontium (Vighi et al., 2019).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mambio.2019.10.004.

References

Aguilar, A., Giménez, J., Gómez-Campos, E., Cardona, L., Borrell, A., 2014. $\delta^{15}N$ value does not reflect fasting in mysticetes. PLoS One 9, e92288, http://dx.doi.org/10.1371/journal.pone.0092288.

Ayliffe, L.K., Cerling, T.E., Robinson, T., West, A.G., Sponheimer, M., Passey, B.H., Hammer, J., Roeder, B., Dearing, M.D., Ehleringer, J.R., 2004. Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. Oecologia 139, 11–22, http://dx.doi.org/10.1007/s00442-003-1479-x.

Bentaleb, I., Martin, C., Vrac, M., Mate, B., Mayzaud, P., Siret, D., De Stephanis, R., Guinet, C., 2011. Foraging ecology of Mediterranean fin whales in a changing environment elucidated by satellite tracking and baleen plate stable isotopes. Mar. Ecol. Prog. Ser. 438, 285–302, http://dx.doi.org/10.3354/meps09269.

Bond, A.L., Hobson, K.A., 2012. Reporting stable-isotope ratios in ecology: recommended terminology, guidelines and best practices. Waterbirds 35, 324–331, http://dx.doi.org/10.1675/063.035.0213.

Bond, A.L., Jardine, T.D., Hobson, K.A., 2016. Multi-tissue stable-isotope analyses can identify dietary specialization. Methods Ecol. Evol. 7, 1428–1437, http://dx.doi.org/10.1111/2041-210X.12620.

Bowen, G.J., Wassenaar, L.I., Hobson, K.A., 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143, 337–348, http://dx.doi.org/10.1007/s00442-004-1813-y.

Busquets-Vass, G., Newsome, S.D., Calambokidis, J., Serra-Valente, G., Jacobsen, J.K., Aguíñiga-García, S., Gendron, D., 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth rates: implications for assessing diet and movement patterns in mysticetes. PLoS One 12, 1–25, http://dx.doi.org/10.1371/journal.pone.0177880.

Clementz, M., Koch, P., 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia 129, 461–472, http://dx.doi.org/10.1007/s004420100745.

DeHart, P.A.P., Picco, C.M., 2015. Stable oxygen and hydrogen isotope analyses of bowhead whale baleen as biochemical recorders of migration and arctic environmental change. Polar Sci. 9, 235–248, http://dx.doi.org/10.1016/j.polar.2015.03.002.

Eisenmann, P., Fry, B., Holyoake, C., Coughran, D., Nicol, S., Bengtson Nash, S., 2016. Isotopic evidence of a wide spectrum of feeding strategies in Southern hemisphere humpback whale baleen records. PLoS One 11, 1–21, http://dx.doi.org/10.1371/journal.pone.0156698.

Fry, B., Arnold, C., 1982. Rapid 13C / 12C turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54, 200–204, http://dx.doi.org/10.1007/BF00378393.

Fudge, D.S., Szewciw, L.J., Schwalb, A.N., 2009. Morphology and development of blue whale baleen: an annotated translation of Tycho Tullberg's classic 1883 paper. Aquat. Mamm. 35, 226–252, http://dx.doi.org/10.1578/AM.35.2.2009.226.

García-Vernet, R., Sant, P., Vikingsson, G.A., Borrell, A., Aguilar, A., 2018. Are stable isotope ratios and oscillations consistent in all baleen plates along the filtering apparatus? Validation of an increasingly used methodology. Rapid Commun. Mass Spectrom. 32, 1257–1262, http://dx.doi.org/10.1002/rcm.8169.

Giménez, J., Gómez-Campos, E., Borrell, A., Cardona, L., Aguilar, A., 2013. Isotopic evidence of limited exchange between Mediterranean and eastern North Atlantic fin whales. Rapid Commun. Mass Spectrom. 27, 1801–1806, http://dx.doi.org/10.1002/rcm.6633.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120, 314–326, http://dx.doi.org/10.1007/s004420050865.

Hobson, K.A., Riget, F.F., Outridge, P.M., Dietz, R., Born, E., 2004. Baleen as a biomonitor of mercury content and dietary history of North Atlantic Minke Whales (*Balaenoptera acutorostrata*): combining elemental and stable isotope approaches. Sci. Total Environ. 331, 69–82, http://dx.doi.org/10.1016/j.scitotenv.2004.03.024.

Hobson, K.A., Schell, D.M., 1998. Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). Can. J. Fish. Aquat. Sci. 55, 2601–2607, http://dx.doi.org/10.1139/cjfas-55-12-2601.

Hunt, K.E., Lysiak, N.S., Moore, M.J., Rolland, R.M., 2016. Longitudinal progesterone profiles in baleen from female North Atlantic right whales (*Eubalaena glacialis*) match known calving history. Conserv. Physiol. 4, 1–9, http://dx.doi.org/10.1093/conphys/cow014.

Matthews, C.J.D., Ferguson, S.H., 2015. Seasonal foraging behaviour of eastern Canada-West Greenland bowhead whales: an assessment of isotopic cycles along baleen. Mar. Ecol. Prog. Ser. 522, 269–286, http://dx.doi.org/10.3354/meps11145.

McMahon, K.W., Hamady, L.L., Thorrold, S.R., 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol. Oceanogr. 58, 697–714.

Mitani, Y., Bando, T., Takai, N., Sakamoto, W., 2006. Patterns of stable carbon and nitrogen isotopes in the baleen of common minke whale *Balaenoptera acutorostrata* from the western North Pacific. Fish. Sci. 72, 69–76, http://dx.doi.org/10.1111/j.1444-2906.2006.01118.x.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18, 293–320, http://dx.doi.org/10.1146/annurev.es.18.110187.001453.

Pfeiffer, C.J., 1992. Cellular structure of terminal baleen in various mysticete species. Aquat. Mamm. 18, 67–73.

Pinto, S.J.D., Shadwick, R.E., 2013. Material and structural properties of fin whale (*Balaenoptera physalus*) zwischensubstanz. J. Morphol. 274, 947–955, http://dx.doi.org/10.1002/jmor.20154.

R Core Team, URL 2016. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.

Rice, D.W., 2002. Baleen. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals. Elsevier-Academic Press, Amsterdam (Netherland), pp. 61–62.

Rita, D., Drago, M., Galimberti, F., Cardona, L., 2017. Temporal consistency of individual trophic specialization in southern elephant seals Mirounga leonina. Mar. Ecol. Prog. Ser. 585, 229–242, http://dx.doi.org/10.3354/meps12411.

Rubenstein, D.R., Hobson, K.A., 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol. Evol. 19, 256–263, http://dx.doi.org/10.1016/j.tree.2004.03.017.

Ryan, C., McHugh, B., Trueman, C.N., Sabin, R., Deaville, R., Harrod, C., Berrow, S.D., O'Connor, I., 2013. Stable isotope analysis of baleen reveals resource partitioning among sympatric rorquals and population structure in fin whales. Mar. Ecol. Prog. Ser. 479, 251–261, http://dx.doi.org/10.3354/meps10231.

Skrzypek, G., 2013. Normalization procedures and reference material selection in stable HCNOS isotope analyses: an overview. Anal. Bioanal. Chem. 405, 2815–2823, http://dx.doi.org/10.1007/s00216-012-6517-2.
Szewciw, L.J., de Kerckhove, D.G., Grime, G.W., Fudge, D.S., 2010. Calcification provides mechanical reinforcement to whale baleen alpha-keratin. Proc. R. Soc. Ser. B, Biol. Sci. 277, 2597–2605, http://dx.doi.org/10.1098/rspb.2010.0399.
Van Utrecht, W.L., 1966. On the growth of the baleen plate of the fin whale and the blue whale. Bijdr. tot Dierkd. 35, 3–38.
Vighi, M., Borrell, A., Víkingsson, G., Gunnlaugsson, T., Aguilar, A., 2019. Strontium in fin whale baleen: a potential tracer of mysticete movements across the oceans? Sci. Total Environ. 650, 1224–1230, http://dx.doi.org/10.1016/j.scitotenv.2018.09.103.
Werth, A.J., Straley, J.M., Shadwick, R.E., 2016. Baleen wear reveals intraoral water flow patterns of mysticete filter feeding. J. Morphol. 277, 453–471, http://dx.doi.org/10.1002/jmor.20510.
Young, S., Deméré, T.A., Ekdale, E.G., Berta, A., Zellmer, N., 2015. Morphometrics and structure of complete baleen racks in gray whales (*Eschrichtius robustus*) from the eastern North Pacific Ocean. Anat. Rec. 298, 703–719, http://dx.doi.org/10.1002/ar.23108.

Amino acid-specific nitrogen stable isotope analysis reveals the winter biology of Icelandic fin whales

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Abstract: The Icelandic stock of fin whales (*Balaenoptera physalus*) aggregate at high latitudes in the South-West of Iceland during summer to feed on krill (mainly *Meganyctiphanes norvegica*). During winter, they migrate to lower latitudes where their trace is lost in subtropical waters. The analysis of bulk nitrogen stable isotopes in the baleen plates of the mysticetes is a useful tool to study the migrations of the whales. However, bulk nitrogen stable isotope values are the result of both, the isotopic signal of the inorganic nitrogen source for the pelagic food web (baseline value) as well as the trophic level of the whale. In this study, both factors were disentangled using amino acid-specific nitrogen isotope values ($\delta^{15}N_{AA}$). Analyses were done with samples from 15 Icelandic fin whales including two different sections of the baleen plates comprising tissues that were synthesised in summer versus those that were synthesised in winter. The $\delta^{15}N_{AA}$ values allowed to robustly quantify the change in trophic level and in baseline $\delta^{15}N$ values between the two seasons. The trophic level increased by 0.63 levels and became more variable in the winter samples, which suggested that the fin whales fed more generalist during winter. Interestingly, the baseline $\delta^{15}N$ values of some whales were very similar in both seasons. Since the baseline $\delta^{15}N$ values, on average, are lower at low latitudes, fin whales with similar summer- and wintertime values either remained in high latitudes during winter or visited areas at lower latitude with locally higher than average $\delta^{15}N$ values, such as upwelling areas or cold eddies. These zones have a high deep-water nitrate concentration and can support a higher primary production than the rest of the open ocean. Both factors, the trophic level and the baseline stable isotopes,

had a similar impact on the bulk stable isotopes values. This showed the importance of considering the two factors when comparing populations that feed in areas with different baseline stable isotopes.

Title: Amino acid-specific nitrogen stable isotope analysis reveals the winter biology of Icelandic fin whales

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Keywords: CSIA; *Balaenoptera physalus*; baleen plate; trophic level; migration.

Abstract

The Icelandic stock of fin whales (*Balaenoptera physalus*) aggregate at high latitudes in the South-West of Iceland during summer to feed on krill (mainly *Meganyctiphanes norvegica*). During winter, they migrate to lower latitudes where their trace is lost in subtropical waters. The analysis of bulk nitrogen stable isotopes in the baleen plates of the mysticetes is a useful tool to study the migrations of the whales. However, bulk nitrogen stable isotope values are the result of both, the isotopic signal of the inorganic nitrogen source for the pelagic food web (baseline value) as well as the trophic level of the whale. In this study, both factors were disentangled using amino acid-specific nitrogen isotope values ($\delta^{15}N_{AA}$). Analyses were done with samples from 15 Icelandic fin whales including two different sections of the baleen plates comprising tissues that were synthesised in summer versus those that were synthesised in winter. The $\delta^{15}N_{AA}$ values allowed to robustly quantify the change in trophic level and in baseline $\delta^{15}N$ values between the two seasons. The trophic level increased by 0.63 levels and became more variable in the winter samples, which suggested that the fin whales fed more generalist during winter. Interestingly, the baseline $\delta^{15}N$ values of some whales were very similar in both seasons. Since the baseline $\delta^{15}N$ values, on average, are lower at low latitudes, fin whales with similar summer- and wintertime values either remained in high latitudes during winter or visited areas at lower latitude with locally higher than average $\delta^{15}N$ values, such as upwelling areas or cold eddies. These zones have a high

deep-water nitrate concentration and can support a higher primary production than the rest of the open ocean. Both factors, the trophic level and the baseline stable isotopes, had a similar impact on the bulk stable isotopes values. This showed the importance of considering the two factors when comparing populations that feed in areas with different baseline stable isotopes.

Introduction

The fin whale (*Balaenoptera physalus*) is a cosmopolite species that aggregates during the summer to feed in highly productive zones (Aguilar and García-Vernet 2018). One of such aggregations meets between the eastern coast of Greenland and the western and southern coast of Iceland (Pike et al. 2019) and forms one of the largest fin whale stocks of the North Atlantic. This stock congregates in the feeding ground in spring and feeds on krill, mainly *Meganyctiphanes norvegica*, during the summer (Sigurjónsson and Víkingsson 1997). Their stay in the Icelandic waters extends well in the autumn when they seem to shift to a capelin based diet (Pike et al. 2019). During winter many fin whales leave the feeding grounds and migrate to their wintering grounds in low latitude zones, although some individuals may remain in the feeding grounds all year round (Mizroch et al. 2009; Soule and Wilcock 2013; Shabangu et al. 2020). Some attempts have been made to study the winter biology of the fin whales using stable isotopes and satellite tags (Silva et al. 2013, 2019; Gauffier et al. 2020; Lydersen et al. 2020), but their location and diet during winter remain largely unknown.

The analysis of the stable isotope ratio of an element (nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) being the most used) is a well-established technique to study migrations (Hobson et al. 2010), especially when metabolically inert tissues are available. While metabolically active tissues are exchanging nutrients with the blood, and therefore replacing their stable isotope ratios at a certain rate, metabolically inert tissues fix the stable isotope ratios once synthesised (Hobson 1999). If this tissue is accumulated, it may provide a time record of the stable isotopes present in the animal during a period. In fin whales, such stable isotope record can be found in the baleen plates (Aguilar et al. 2014; García-Vernet et al. 2018), where the $\delta^{15}N$ values form a characteristic sinusoidal pattern that can be related to the annual cycle of the whales (Best and Schell 1996; Hobson and Schell 1998; deHart and Picco 2015; Busquets-Vass et al. 2017; Sensor et al. 2018).

The stable isotope ratios of an animal largely depend on the stable isotope ratios of the environment where it lives, i.e. the baseline stable isotope values (McMahon et al. 2013). However, they are modulated by the tissue discrimination factor (Borrell et al. 2012), the trophic level (Post 2002) and by some physiological functions, such as reproduction and growth (Warinner and Tuross 2010; Borrell et al. 2016). All these factors intermingle to produce characteristic stable isotope signatures for each individual, tissue and phase of the life cycle. Thus, it is often impossible to know which

factor, or in which proportion, is responsible for the changes of the $\delta^{15}N$ values in a tissue. Specifically, it is difficult to know if a change in $\delta^{15}N$ is due to a change in baseline value or a change in diet, and amino acid-specific stable isotope analysis (CSIA-AA) has been proposed as a solution to this issue.

CSIA-AA analyses the stable isotopes from specific amino acids. Since different amino acids are affected differently by the factors mentioned before, their stable isotopes can be used to establish the proportion in which each factor is affecting the stable isotopes of the bulk tissue (Matthews and Ferguson 2013; Ruiz-Cooley et al. 2014; Brault et al. 2019). Phenylalanine (Phe), for example, is considered a "source" amino acid because it cannot be synthesised by animals and therefore its $\delta^{15}N$ value ($\delta^{15}N_{phe}$ values) remains largely unchanged through the trophic web (McClelland and Montoya 2002; Popp et al. 2007). Others amino acids, such as glutamic acid (Glu), proline (Pro) or alanine (Ala), are considered "trophic" amino acids because their stable isotope ratios change substantially from a trophic level to the next (McClelland and Montoya 2002; Popp et al. 2007). Thus, $\delta^{15}N_{phe}$ values can be used to establish the baseline $\delta^{15}N$ values, and the difference between the $\delta^{15}N$ values of a trophic amino acid and those of Phe can be used to establish the trophic level of the organism (McClelland and Montoya 2002; Chikaraishi et al. 2009).

The current study aimed to establish the proportion in which the trophic level and the $\delta^{15}N$ baseline value affect the stable isotope ratios of Icelandic fin whale during their migration. Baleen plates were analysed using CSIA-AA to measure the $\delta^{15}N$ baseline values and the trophic level of the whales during summer and winter. These results provided information on the winter biology of the fin whale and may help the interpretation of future studies using stable isotope analysis. Finally, ecosystem information, such as the trophic enrichment factor (i.e. the increase in bulk $\delta^{15}N$ values in every trophic level) was also calculated for each season.

Methods

Sample Collection

The baleen plates were collected from 15 Icelandic fin whales flensed at the Hvalur H/F whaling station (Hvalfjordur, Iceland). Each baleen plate was excised from the middle section of the right jaw at the very proximal end, in contact with the jawbone, to ensure that the whole plate had been extracted. The "gum" of the plate was removed using a scalpel and the plate surface was rinsed using chloroform/methanol (2:1) solution. Baleen sampling was conducted by scratching the surface of the plate with a Dremel 300 series every 1 cm along the first 40 cm of the plate.

Bulk and compound-specific stable isotopes analysis

All samples were analysed for bulk stable isotope ratios at the Scientific and Technological Centre (CCiT) of the University of Barcelona and have been previously reported in Garcia-Vernet et al, (eventually). Nitrogen stable isotope abundances are expressed in delta (δ) notation, with relative variations of stable isotope ratios expressed in per mil (‰) deviations from predefined international standards (Bond and Hobson 2012). The international standards used were atmospheric nitrogen (air) for $\delta^{15}N$. However, the data were normalized using commercially available laboratory reference materials. Secondary isotopic reference materials of known ^{15}N:^{14}N ratios, as given by the International Atomic Energy Agency (IAEA, Vienna, Austria), were used for calibration at a precision of 0.05‰. These materials included $(NH_4)_2SO_4$ (IAEA-N-1, $\delta^{15}N$ = +0.4 ‰ and IAEA-N-2, $\delta^{15}N$ = +20.3 ‰), L-glutamic acid (IAEA USGS-40, $\delta^{15}N$= -4.5 ‰) and KNO_3 (IAEA-N-3, $\delta^{15}N$= +4.7 ‰). These isotopic reference materials were assessed once per 12 analysed samples to recalibrate the system and compensate for any measurement drift over time. The raw data were normalized by the multipoint normalization method based on linear regression (Skrzypek 2013).

The results of the bulk stable isotope analyses showed the classical oscillation pattern caused by the seasonal migration of this species (Garcia-Vernet, eventually). This pattern was used to choose, from each plate, one sample corresponding to the winter season (maximum $\delta^{15}N$) and one sample corresponding to the adjacent summer season (minimum $\delta^{15}N$). This decision was based on the decrease tendency in the first centimetres of most of the baleen plates from this population (Garcia-Vernet, eventually) and in the inverse correlation between the fin whale skin $\delta^{15}N$ values and the day of capture (unpublished data). Both tendencies suggest that the fin whales' bulk $\delta^{15}N$ values were higher during winter and decreased during summer.

These 30 samples were analysed for amino acid nitrogen-specific isotope ratios in the Leibniz Institute for Baltic Sea Research. These samples were hydrolysed with hydrochloric acid and derivatized to trifluoro-acetylated isopropyl amino acid esters (AA-TFA/IP; Hofmann et al. 2003) and cleaned as described by Veuger et al. (2005). The amino acid nitrogen-specific isotope analyses were performed by a Thermo MAT 253 isotope ratio mass spectrometer (IRMS) coupled to a Thermo Trace GC 1310 gas chromatograph (GC) fitted with a 5% phenyl polysilphenylene siloxane non-polar column (BPX-5, 60 m, 0.32 mm inner diameter, film thickness 1.0 µm, Scientific Glass Engineering Analytical Science, Ringwood, Victoria, Australia). The combustion unit was a Thermo Isolink fixed at the GC oven and connected to the IRMS via a ConFlo IV interface.

Trophic level

$\delta^{15}N$ values of a source amino acid ($\delta^{15}N_{source}$) were used in combination with the $\delta^{15}N$ values of a trophic amino acid ($\delta^{15}N_{trophic}$) to calculate the trophic level (Chikaraishi et al. 2009). As source amino acid, we used the $\delta^{15}N$ value of phenylalanine (McClelland

and Montoya 2002). Up until recently, Glu has been used as the trophic amino acid because its trophic discrimination factor had a low variability in low trophic level species (Chikaraishi et al. 2009; Nielsen et al. 2015). However, the estimates obtained with this amino acid are not reliable in marine mammals and other top predator species (Lorrain et al. 2009; Dale et al. 2011; Germain et al. 2013). For this reason, Pro was used as the trophic amino acid to estimate trophic level using the following equation.

$$TL = \frac{\delta^{15}N_{Pro} - \delta^{15}N_{Phe} - \beta}{TDF} + 1$$

where β is the difference between the δ^{15}N values of the two amino acids at the autotrophs level (3.1 ‰, Chikaraishi et al. 2009) and TDF is the trophic discrimination factor between Pro and Phe at each trophic level (4.5 ‰, McMahon and McCarthy 2016; Brault et al. 2019).

Statistical analysis

All statistical analyses were carried out using the free software R (R Core Team 2020) and the software Rstudio (RStudio Team 2016). The effects of δ^{15}N baseline values ($\delta^{15}N_{Phe}$ values) and trophic level on the bulk values were estimated using generalized linear models (glm). The relative importance of each of these two factors was estimated using the R package *relaimpo* (Grömping 2006). Normality of the residuals was tested using the Shapiro-Wilk test and the model was visually inspected using the "residuals Vs fitted values" plot.

T-tests were used to compare the differences between summer and winter of three response variables: bulk δ^{15}N values, trophic level and $\delta^{15}N_{Phe}$ values. Normality and homoscedasticity were tested using the Shapiro-Wilk test and the Bartlett test.

The regression between the baseline-corrected bulk δ^{15}N (i.e. bulk δ^{15}N – $\delta^{15}N_{Phe}$) and the trophic level was used to calculate the trophic enrichment factor of the summer and winter ecosystems (Mompeán et al. 2016). The slope of this regression represents the increase in bulk δ^{15}N values with every trophic level.

Results

The bulk δ^{15}N values were significantly correlated to the trophic level (t= 11.74, d.f. = 1,27, p-value < 0.001) and to the $\delta^{15}N_{Phe}$ values (t= 10.72, d.f. = 1,27, p-value < 0.001) and the model could explain 86% of the deviance. The relative importance of each factor was: 49% trophic level and 37% $\delta^{15}N_{Phe}$ values.

The bulk tissue δ^{15}N values were statistically different (t-value = -9.3, df = 14, p-value <0.001) between the summer (mean ±SD: 8.14 ±1.03 ‰) and winter (10.72 ±0.98 ‰). The trophic level (Fig. 1) of the studied animals statistically increased from 3.00 ±0.39 in summer to 3.63 ±0.67 (t-value = -3.5, df = 14, p-value = 0.004). $\delta^{15}N_{Phe}$ values (Fig. 2) were also higher in winter (5.23 ±2.4 ‰) than in summer (4 ±2.14 ‰), but

the differences were not statistically significant (t-value = -1.82, df = 14, p-value = 0.089). It is worth noting that the standard deviations were not statistically different in any of the cases, but the trophic level standard deviation was twice as high in winter than in summer. Detailed results can be found in the Supplementary Material.

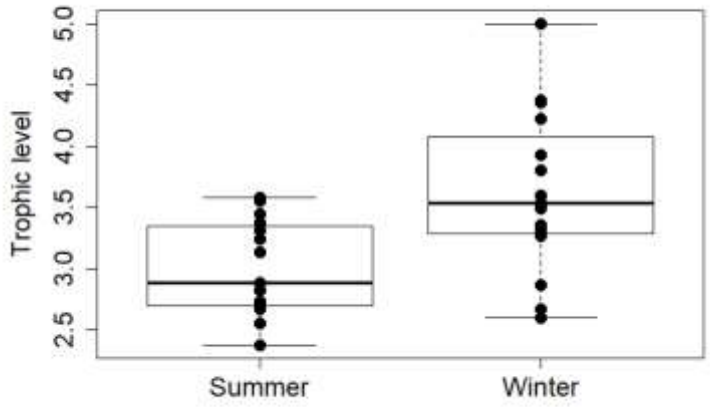

Figure 1. Seasonal change of trophic level in Icelandic fin whale.

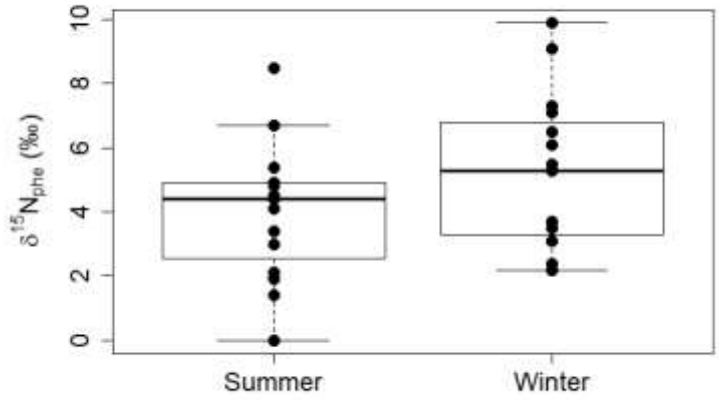

Figure 2. Seasonal change of $\delta^{15}N_{phe}$ in Icelandic fin whales

The trophic enrichment factor was not statistically different between seasons (t-value =-0.83; d.f.= 1, 26; p-value = 0.41). Its value in both seasons was 3.54 ±0.32 ‰·per trophic level (Fig. 3).

Discussion

The average bulk tissue stable isotope values were higher during winter than during summer. This change could have been produced by a change in location or a change in trophic level, and the CSIA-AA analysis allowed to determine the relative importance of each factor. The stable isotopes ratios in the bulk tissue were highly

correlated with the $\delta^{15}N_{Phe}$ values and the trophic level of the whale. Although these results were expected, it is worth noting that the two factors had similar relative importance affecting the bulk stable isotopes. Bulk tissue $\delta^{15}N$ values are often used as a proxy of the trophic level of the organism, but these results may be misleading if a possible change in baseline is not considered. In migrating species, the effect of a change in isotopic baseline may be as important as the effect of a change in trophic level, and both should be regarded in the interpretation of the results.

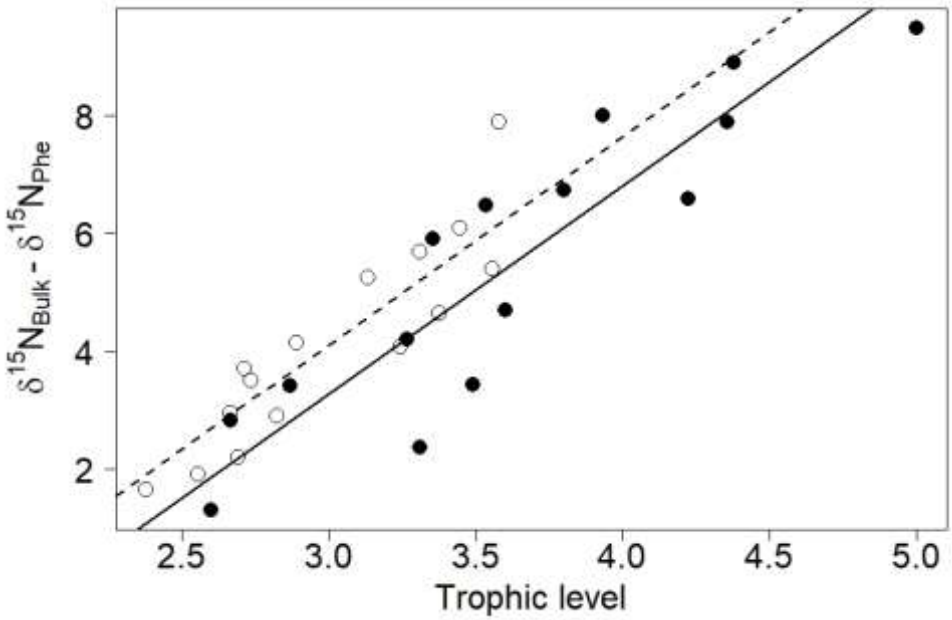

Figure 3. Correlation between the baseline-corrected bulk $\delta^{15}N$ values

The trophic level of the sampled fin whales during summer was consistent with diet based on herbivorous zooplankton, which is in accordance with previous studies (Sigurjónsson and Víkingsson 1997). However, during winter their average trophic level increased, which could be explained by two reasons. First, fin whales could switch from a zooplankton-based diet to a more diverse diet that included schooling fish, as other fin whale stocks at the end of summer in the North Atlantic (Christensen et al. 1992; Skern-Mauritzen et al. 2011; Gavrilchuk et al. 2014; Ryan et al. 2014; Nøttestad et al. 2015; Pike et al. 2019). Second, fin whales could still be feeding on krill, which become omnivorous or carnivorous during winter (Schmidt 2010). These two hypotheses are not exclusive, and the high variability of the trophic level during winter suggest that different whales may be using different strategies.

The differences in $\delta^{15}N_{Phe}$ values between the two seasons were not statistically significant, but they were close to the statistical threshold. It is possible that the high variability of the data hid the actual differences in the statistic and that a larger sample size would have shown that the $\delta^{15}N_{Phe}$ value in winter was higher than in summer.

Either way, it was surprising to find similar, or even higher, $\delta^{15}N_{Phe}$ values in winter and summer rather than lower $\delta^{15}N_{Phe}$ values. Although some fin whales remain at high latitudes during winter (Mizroch et al. 2009; Soule and Wilcock 2013; Shabangu et al. 2020), most of the population migrate to lower latitudes (Silva et al. 2013; Lydersen et al. 2020; Pereira et al. 2020). Here, the baseline $\delta^{15}N$ values may be expected to be lower than in high latitudes since the N_2 fixation in these largely oligotrophic waters causes low $\delta^{15}N$ values (0-6 ‰ in tropical and subtropical zooplankton as opposed to ~8 ‰ in high latitude zooplankton) (McClelland et al. 2003; McMahon et al. 2013). Thus, a lower $\delta^{15}N_{Phe}$ may be expected during winter than during summer. However, the $\delta^{15}N_{Phe}$ values also may be expected to increase on a regional scale due to the emergence of deep-water nitrate. These areas, such as cold eddies and upwelling zones, have enhanced $\delta^{15}N$ values compared to areas with greater proportion of N2 fixation; in some extreme cases, denitrification in this areas rises the $\delta^{15}N$ values even further, such as in the Benguela upwelling area, where $\delta^{15}N$ values of 5 ‰ above the global mean occur (Nagel et al, 2013). Many of the sampled whales could use such areas, but the high variability in the winter $\delta^{15}N_{Phe}$ values suggests that some individuals may still use more oligotrophic sites.

Upwelling zones are enriched in deep water nitrate and can support higher primary productivity than those zones that rely on the fixation of molecular nitrogen (McGillicuddy et al. 2003). Although many of these mesoscale features tend to be temporal (Chelton et al. 2011), their effects are long enough to attract high trophic level organisms (Godø et al. 2012), including marine mammals (Davis et al. 2002; Campagna et al. 2006; Bailleul et al. 2010; Baines and Reichelt 2014; Garcia-Rojas et al. 2018). Fin whales from other stocks have already been associated to these oceanographic features (Baines et al. 2017; Lydersen et al. 2020; Pérez-Jorge et al. 2020) and our results suggest that part of the Islandic fin whales may also be using these areas to feed.

On top of the two fin whale characteristics already discussed, the CSIA-AA also provided characteristics from the ecosystem where the fin whales fed, such as the trophic enrichment factor. The trophic enrichment factor describes the increase in bulk $\delta^{15}N$ values correspondent to the increase of one trophic level. It often used in bulk $\delta^{15}N$ studies to estimate the trophic level of the species (Post 2002; Chikaraishi et al. 2015) or as a correction factor to determine the dietary quality of a species (Cardona et al. 2012; Ryan et al. 2014). The trophic enrichment factor is tissue- and species-specific, and should be estimated in control-fed experiments in order to apply it to diet studies (Hobson et al. 1996; Hussey et al. 2010; Borrell et al. 2012). However, the ecosystem average trophic enrichment factor can be used as a rough estimator of the trophic level of an individual when the baseline bulk $\delta^{15}N$ is known. The average trophic enrichment factor of the ecosystems where the fin whales fed was 3.54 ±0.32 ‰·trophic level^{-1} in both seasons, which is similar to the trophic discrimination obtained in SIA studies (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001) and CSIA-AA studies

(Mompeán et al. 2016). Our results support the use of this value to estimate the trophic level as long as the baseline $\delta^{15}N$ values are taken into account (Bas et al. 2020).

Finally, it is worth mentioning that the trophic level calculated using CSIA-AA is heavily dependent on the amino acids and the coefficients used. The most used trophic amino acid is Glu, which provides accurate trophic levels in low trophic level species but tends to underestimate it in high trophic level species (Lorrain et al. 2009; Dale et al. 2011; Germain et al. 2013; McMahon and McCarthy 2016). Different reasons have been proposed for this bias, such as the excretion method of the species (Germain et al. 2013) or the protein quality of the diet (Hoen et al. 2014; Chikaraishi et al. 2015; McMahon et al. 2015). On the other hand, Pro seems to be independent to these biases and provides trophic levels with low precision but high accuracy across the whole trophic web (McMahon and McCarthy 2016; Brault et al. 2019). The tropic levels measured in this study using Pro provided an accurate trophic level during summer when the diet of the species is well known (Sigurjónsson and Víkingsson 1997), and a trophic enrichment factor that agrees with those found in several other studies (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001; Mompeán et al. 2016). Thus, the use of Pro as trophic amino acid is encouraged in future studies using CSIA-AA, especially if they analyse high trophic level species.

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References

Aguilar, A., and R. García-Vernet. 2018. Fin Whale (Balaenoptera physalus), p. 368–371. *In* B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.], Encyclopedia of Marine Mammals. Elsevier.

Aguilar, A., J. Giménez, E. Gómez-Campos, L. Cardona, and A. Borrell. 2014. $\delta^{15}N$ value does not reflect fasting in mysticetes. PLoS One **9**: e92288. doi:10.1371/journal.pone.0092288

Bailleul, F., C. Cotté, and C. Guinet. 2010. Mesoscale eddies as foraging area of a deep-diving predator, the southern elephant seal. Mar. Ecol. Prog. Ser. **408**: 251–264. doi:10.3354/meps08560

Baines, M. E., and M. Reichelt. 2014. Upwellings, canyons and whales: An important winter habitat for balaenopterid whales off Mauritania, northwest Africa. J. Cetacean Res. Manag. **14**: 57–67.

Baines, M., M. Reichelt, and D. Griffin. 2017. An autumn aggregation of fin (Balaenoptera physalus) and blue whales (B. musculus) in the Porcupine Seabight, southwest of Ireland. Deep. Res. Part II Top. Stud. Oceanogr. **141**: 168–177. doi:10.1016/j.dsr2.2017.03.007

Bas, M., M. Salemme, E. J. Green, F. Santiago, C. Speller, M. Álvarez, I. Briz i Godino, and L. Cardona. 2020. Predicting habitat use by the Argentine hake Merluccius hubbsi in a warmer world: inferences from the Middle Holocene. Oecologia **193**: 461–474. doi:10.1007/s00442-020-04667-z

Best, P. B., and D. M. Schell. 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar. Biol. **124**: 483–494. doi:10.1007/BF00351030

Bond, A. L., and K. A. Hobson. 2012. Reporting stable-isotope ratios in ecology: recommended terminology, guidelines and best practices. Waterbirds **35**: 324–331. doi:10.1675/063.035.0213

Borrell, A., N. Abad-Oliva, E. Gómez-Campos, J. Giménez, and A. Aguilar. 2012. Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. Rapid Commun. Mass Spectrom. **26**: 1596–1602. doi:10.1002/rcm.6267

Borrell, A., E. Gómez-Campos, and A. Aguilar. 2016. Influence of reproduction on stable isotope ratios: nitrogen and carbon isotope discrimination between mothers, fetuses, and milk in the fin whale, a capital breeder. Physiol. Biochem. Zool. **89**: 41–50. doi:10.1086/684632

Brault, E. K., P. L. Koch, D. P. Costa, and others. 2019. Trophic position and foraging ecology of Ross, Weddell, and crabeater seals revealed by compound-specific isotope analysis. Mar. Ecol. Prog. Ser. **611**: 1–18. doi:10.3354/meps12856

Busquets-Vass, G., S. D. Newsome, J. Calambokidis, G. Serra-Valente, J. K. Jacobsen, S. Aguíñiga-García, and D. Gendron. 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth rates: Implications for assessing diet and movement patterns in mysticetes. PLoS One **12**: 1–25. doi:https://doi.org/10.1371/journal.pone.0177880

Campagna, C., A. R. Piola, M. Rosa Marin, M. N. Lewis, and T. Fernández. 2006. Southern elephant seal trajectories, fronts and eddies in the Brazil/Malvinas Confluence.

Deep. Res. Part I Oceanogr. Res. Pap. **53**: 1907–1924. doi:10.1016/j.dsr.2006.08.015

Cardona, L., I. Á. de Quevedo, A. Borrell, and A. Aguilar. 2012. Massive consumption of gelatinous plankton by mediterranean apex predators. PLoS One **7**. doi:10.1371/journal.pone.0031329

Chelton, D. B., M. G. Schlax, and R. M. Samelson. 2011. Global observations of nonlinear mesoscale eddies. Prog. Oceanogr. **91**: 167–216. doi:10.1016/j.pocean.2011.01.002

Chikaraishi, Y., N. O. Ogawa, Y. Kashiyama, and others. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol. Oceanogr. Methods **7**: 740–750. doi:10.4319/lom.2009.7.740

Chikaraishi, Y., S. A. Steffan, Y. Takano, and N. Ohkouchi. 2015. Diet quality influences isotopic discrimination among amino acids in an aquatic vertebrate. Ecol. Evol. **5**: 2048–2059. doi:10.1002/ece3.1491

Christensen, I., T. Haug, and N. Oien. 1992. A review of feeding and reproduction in large baleen whales (Mysticeti) and sperm whales Physeter macrocephalus in Norwegian and adjacent waters. Fauna Nor. Ser. A **13**: 39–48.

Dale, J. J., N. J. Wallsgrove, B. N. Popp, and K. N. Holland. 2011. Nursery habitat use and foraging ecology of the brown stingray Dasyatis lata determined from stomach contents, bulk and amino acid stable isotopes. Mar. Ecol. Prog. Ser. **433**: 221–236. doi:10.3354/meps09171

Davis, R. W., J. G. Ortega-Ortiz, C. A. Ribic, and others. 2002. Cetacean habitat in the northern oceanic Gulf of Mexico. Deep. Res. Part I Oceanogr. Res. Pap. **49**: 121–142. doi:10.1016/S0967-0637(01)00035-8

deHart, P. A. P., and C. M. Picco. 2015. Stable oxygen and hydrogen isotope analyses of bowhead whale baleen as biochemical recorders of migration and arctic environmental change. Polar Sci. **9**: 235–248. doi:10.1016/j.polar.2015.03.002

Garcia-Rojas, M. I., K. C. S. Jenner, P. C. Gill, M. N. M. Jenner, A. L. Sutton, and R. D. McCauley. 2018. Environmental evidence for a pygmy blue whale aggregation area in the Subtropical Convergence Zone south of Australia. Mar. Mammal Sci. **34**: 901–923. doi:10.1111/mms.12494

García-Vernet, R., P. Sant, G. A. Vikingsson, A. Borrell, and A. Aguilar. 2018. Are stable isotope ratios and oscillations consistent in all baleen plates along the filtering

apparatus? Validation of an increasingly used methodology. Rapid Commun. Mass Spectrom. **32**: 1257–1262. doi:10.1002/rcm.8169

Gauffier, P., A. Borrell, M. A. Silva, and others. 2020. Wait your turn, North Atlantic fin whales share a common feeding ground sequentially. Mar. Environ. Res. **155**: 104884. doi:10.1016/J.MARENVRES.2020.104884

Gavrilchuk, K., V. Lesage, C. Ramp, R. Sears, M. Bérubé, S. Bearhop, and G. Beauplet. 2014. Trophic niche partitioning among sympatric baleen whale species following the collapse of groundfish stocks in the Northwest Atlantic. Mar. Ecol. Prog. Ser. **497**: 285–301. doi:10.3354/meps10578

Germain, L. R., P. L. Koch, J. Harvey, and M. D. McCarthy. 2013. Nitrogen isotope fractionation in amino acids from harbor seals: Implications for compound-specific trophic position calculations. Mar. Ecol. Prog. Ser. **482**: 265–277. doi:10.3354/meps10257

Godø, O. R., A. Samuelsen, G. J. Macaulay, R. Patel, S. S. Hjøllo, J. Horne, S. Kaartvedt, and J. A. Johannessen. 2012. Mesoscale eddies are oases for higher trophic marine life. PLoS One **7**: 1–9. doi:10.1371/journal.pone.0030161

Grömping, U. 2006. Relative importance for linear regression in R: the package relaimpo. J. Stat. Softw. **17**: 1–27.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: A review. Oecologia **120**: 314–326. doi:10.1007/s004420050865

Hobson, K. A., R. Barnett-Johnson, and T. Cerling. 2010. Using Isoscapes to Track Animal Migration, p. 273–298. *In* J.B. West, G.J. Bowen, T.E. Dawson, and K.P. Tu [eds.], Isoscapes: Understanding movement, pattern, and process on Earth through isotope mapping. Springer Netherlands.

Hobson, K. A., and D. M. Schell. 1998. Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). Can. J. Fish. Aquat. Sci. **55**: 2601–2607. doi:10.1139/cjfas-55-12-2601

Hobson, K. A., D. M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive seals: implications for dietary reconstruction. Can. J. Fish. Aquat. Sci. **53**: 528–533. doi:10.1139/f95-209

Hoen, D. K., S. L. Kim, N. E. Hussey, N. J. Wallsgrove, J. C. Drazen, and B. N. Popp. 2014. Amino acid 15N trophic enrichment factors of four large carnivorous fishes. J. Exp. Mar. Bio. Ecol. **453**: 76–83. doi:10.1016/j.jembe.2014.01.006

Hofmann, D., M. Gehre, and K. Jung. 2003. Sample preparation techniques for the determination of natural 15N/14N variations in amino acids by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Isotopes Environ. Health Stud. **39**: 233–244. doi:10.1080/1025601031000147630

Hussey, N. E., J. Brush, I. D. McCarthy, and A. T. Fisk. 2010. δ15N and δ13C diet-tissue discrimination factors for large sharks under semi-controlled conditions. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. **155**: 445–453. doi:10.1016/j.cbpa.2009.09.023

Lorrain, A., B. Graham, F. Ménard, B. Popp, S. Bouillon, P. Van Breugel, and Y. Cherel. 2009. Nitrogen and carbon isotope values of individual amino acids: A tool to study foraging ecology of penguins in the Southern Ocean. Mar. Ecol. Prog. Ser. **391**: 293–306. doi:10.3354/meps08215

Lydersen, C., J. V. Garcia, M. P. Heide-Jørgensen, N. Øien, C. Guinet, and K. M. Kovacs. 2020. Autumn movements of fin whales (*Balaenoptera physalus*) from Svalbard, Norway, revealed by satellite tracking. Sci. Rep. **10**. doi:10.1038/s41598-020-73996-z

Matthews, C. J. D., and S. H. Ferguson. 2013. Spatial segregation and similar trophic-level diet among eastern Canadian Arctic/north-west Atlantic killer whales inferred from bulk and compound specific isotopic analysis. J. Mar. Biol. Assoc. United Kingdom **94**: 1343–1355. doi:10.1017/S0025315413001379

McClelland, J. W., C. M. Holl, and J. P. Montoya. 2003. Relating low δ15N values of zooplankton to N 2-fixation in the tropical North Atlantic: Insights provided by stable isotope ratios of amino acids. Deep. Res. Part I Oceanogr. Res. Pap. **50**: 849–861. doi:10.1016/S0967-0637(03)00073-6

McClelland, J. W., and J. P. Montoya. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. Ecology **83**: 2173–2180. doi:10.1890/0012-9658(2002)083[2173:TRATNI]2.0.CO;2

McGillicuddy, D. J., L. A. Anderson, S. C. Doney, and M. E. Maltrud. 2003. Eddy-driven sources and sinks of nutrients in the upper ocean: Results from a 0.1° resolution model of the North Atlantic. Global Biogeochem. Cycles **17**: n/a-n/a. doi:10.1029/2002gb001987

McMahon, K. W., L. L. Hamady, and S. R. Thorrold. 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol. Oceanogr. **58**: 697–714. doi:10.4319/lo.2013.58.2.0697

McMahon, K. W., and M. D. McCarthy. 2016. Embracing variability in amino acid δ15N fractionation: Mechanisms, implications, and applications for trophic ecology. Ecosphere **7**: 1–26. doi:10.1002/ecs2.1511

McMahon, K. W., S. R. Thorrold, T. S. Elsdon, and M. D. Mccarthy. 2015. Trophic discrimination of nitrogen stable isotopes in amino acids varies with diet quality in a marine fish. Limnol. Oceanogr. **60**: 1076–1087. doi:10.1002/lno.10081

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. Geochim. Cosmochim. Acta **48**: 1135–1140. doi:10.1016/0016-7037(84)90204-7

Mizroch, S. A., D. W. Rice, D. Zwiefelhofer, J. Waite, and W. L. Perryman. 2009. Distribution and movements of fin whales in the North Pacific Ocean. Mamm. Rev. **39**: 193–227. doi:10.1111/j.1365-2907.2009.00147.x

Mompeán, C., A. Bode, E. Gier, and M. D. McCarthy. 2016. Bulk vs. amino acid stable N isotope estimations of metabolic status and contributions of nitrogen fixation to size-fractionated zooplankton biomass in the subtropical N Atlantic. Deep. Res. Part I Oceanogr. Res. Pap. **114**: 137–148. doi:10.1016/j.dsr.2016.05.005

Nielsen, J. M., B. N. Popp, and M. Winder. 2015. Meta-analysis of amino acid stable nitrogen isotope ratios for estimating trophic position in marine organisms. Oecologia **178**: 631–642. doi:10.1007/s00442-015-3305-7

Nøttestad, L., B. A. Krafft, V. Anthonypillai, M. Bernasconi, L. Langård, H. L. Mørk, and A. Fernö. 2015. Recent changes in distribution and relative abundance of cetaceans in the Norwegian Sea and their relationship with potential prey. Front. Ecol. Evol. **2**. doi:10.3389/fevo.2014.00083

Pereira, A., D. Harris, P. L. Tyack, and L. Matias. 2020. Fin whale acoustic presence and song characteristics in seas to the southwest of Portugal. J. Acoust. Soc. Am. **147**: 2235–2249. doi:10.1121/10.0001066

Pérez-Jorge, S., M. Tobeña, R. Prieto, F. Vandeperre, B. Calmettes, P. Lehodey, and M. A. Silva. 2020. Environmental drivers of large-scale movements of baleen whales in the mid-North Atlantic Ocean. Divers. Distrib. **26**: 683–698. doi:10.1111/ddi.13038

Pike, D. G., T. Gunnlaugsson, B. Mikkelsen, S. D. Halldórsson, and G. Víkingsson. 2019. Estimates of the abundance of cetaceans in the central North Atlantic from the NASS Icelandic and Faroese shipboard surveys conducted in 2015. NAMMCO Sci. Publ. **11**. doi:10.7557/3.4941

Popp, B. N., B. S. Graham, R. J. Olson, C. C. S. Hannides, M. J. Lott, G. A. López-Ibarra, F. Galván-Magaña, and B. Fry. 2007. Insight into the Trophic Ecology of Yellowfin Tuna, Thunnus albacares, from Compound-Specific Nitrogen Isotope Analysis of Proteinaceous Amino Acids. Terr. Ecol. **1**: 173–190. doi:10.1016/S1936-7961(07)01012-3

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**: 703–718. doi:Doi 10.2307/3071875

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RStudio Team. 2016. RStudio: integrated development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Ruiz-Cooley, R. I., P. L. Koch, P. C. Fiedler, and M. D. McCarthy. 2014. Carbon and nitrogen isotopes from top predator amino acids reveal rapidly shifting ocean biochemistry in the outer California current. PLoS One **9**. doi:10.1371/journal.pone.0110355

Ryan, C., S. D. Berrow, B. Mchugh, C. O'Donnell, C. N. Trueman, and I. O'Connor. 2014. Prey preferences of sympatric fin (Balaenoptera physalus) and humpback (Megaptera novaeangliae) whales revealed by stable isotope mixing models. Mar. Mammal Sci. **30**: 242–258. doi:10.1111/mms.12034

Schmidt, K. 2010. Food and Feeding in Northern Krill (Meganyctiphanes norvegica Sars), p. 127–171. *In* G.A. Tarling [ed.], Advances in Marine Biology. Elsevier Ltd.

Sensor, J. D., J. C. George, M. T. Clementz, and others. 2018. Age estimation in bowhead whales using tympanic bulla histology and baleen isotopes. Mar. Mammal Sci. **34**: 347–364. doi:10.1111/mms.12476

Shabangu, F., R. Andrew, D. Yemane, and K. Findlay. 2020. Acoustic seasonality, behaviour and detection ranges of Antarctic blue and fin whales under different sea ice conditions off Antarctica. Endanger. Species Res. **43**: 21–37. doi:10.3354/esr01050

Sigurjónsson, J., and G. A. Víkingsson. 1997. Seasonal abundance of and estimated food consumption by cetaceans in Icelandic and adjacent waters. J. Northwest Atl. Fish. Sci. **22**: 271–287. doi:10.2960/J.v22.a20

Silva, M. A., A. Borrell, R. Prieto, P. Gauffier, M. Bérubé, P. J. Palsbøl, and A. Colaço. 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. R. Soc. Open Sci. **6**. doi:10.1098/rsos.181800

Silva, M. A., R. Prieto, I. Jonsen, M. F. Baumgartner, and R. S. Santos. 2013. North Atlantic blue and fin whales suspend their spring migration to forage in middle latitudes: building up energy reserves for the journey? PLoS One **8**. doi:10.1371/journal.pone.0076507

Skern-Mauritzen, M., E. Johannesen, A. Bjørge, and N. Øien. 2011. Baleen whale distributions and prey associations in the Barents Sea. Mar. Ecol. Prog. Ser. **426**: 289–301. doi:10.3354/meps09027

Skrzypek, G. 2013. Normalization procedures and reference material selection in stable HCNOS isotope analyses: An overview. Anal. Bioanal. Chem. **405**: 2815–2823. doi:10.1007/s00216-012-6517-2

Soule, D. C., and W. S. D. Wilcock. 2013. Fin whale tracks recorded by a seismic network on the Juan de Fuca Ridge, Northeast Pacific Ocean. J. Acoust. Soc. Am. **133**: 1751–1761. doi:10.1121/1.4774275

Veuger, B., J. J. Middelburg, H. T. S. Boschker, and M. Houtekamer. 2005. Analysis of 15N incorporation into D-alanine: A new method for tracing nitrogen uptake by bacteria. Limnol. Oceanogr. Methods **3**: 230–240. doi:10.4319/lom.2005.3.230

Warinner, C., and N. Tuross. 2010. Brief communication: Tissue isotopic enrichment associated with growth depression in a pig: Implications for archaeology and ecology. Am. J. Phys. Anthropol. **141**: 486–493. doi:10.1002/ajpa.21222

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. Limnol. Oceanogr. **46**: 2061–2066. doi:10.4319/lo.2001.46.8.2061

Capítulo 3

Earplugs provide insight into possible specialisation in fin whales (*Balaenoptera physalus*)

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Abstract: Individual specialisation can be an advantageous strategy in which the individuals of one population occupy a small portion of the population niche. In some species, the individuals are generalist during their youth and become individual specialists at some point of their development. Individual specialists may find it difficult to adapt to a change in the environment if they cannot revert their strategy. This would be especially important in long lived animals because they are more likely to encounter such a change. To detect a change in the individual specialization strategy in a long-lived species, nitrogen and carbon stable isotope ratios were analysed in the earplugs of 17 fin whales (*Balaenoptera physalus*). The individual specialisation index was measured for the entire life of the individuals and, when possible, for three life periods: 4-8 years, 9-13 years and 14-18 years. A bootstrap analysis was used to detect the threshold index value under which a whale can be considered individual specialist. 76% of the fin whales were individual specialists when their entire life was considered. When only the 5-year periods were considered, it was found that several whales appeared to vary their degree of individual specialization between periods.

Title: Earplugs provide insight into possible specialisation in fin whales (*Balaenoptera physalus*)

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Abstract

Individual specialisation can be an advantageous strategy in which the individuals of one population occupy a small portion of the population niche. In some species, the individuals are generalist during their youth and become individual specialists at some point of their development. Individual specialists may find it difficult to adapt to a change in the environment if they cannot revert their strategy. This would be especially important in long lived animals because they are more likely to encounter such a change. To detect a change in the individual specialization strategy in a long-lived species, nitrogen and carbon stable isotope ratios were analysed in the earplugs of 17 fin whales (*Balaenoptera physalus*). The individual specialisation index was measured for the entire life of the individuals and, when possible, for three life periods: 4-8 years, 9-13 years and 14-18 years. A bootstrap analysis was used to detect the threshold index value under which a whale can be considered individual specialist. 76% of the fin whales were individual specialists when their entire life was considered. When only the 5-year periods were considered, it was found that several whales appeared to vary their degree of individual specialization between periods.

Introduction

Many generalist populations are indeed composed of a mixture of specialised individuals, which occupy only a narrow portion of the population ecological niche (e.g. Bogusch et al., 2006; Hoelzel et al., 1989; Hückstädt et al., 2012; Rita et al., 2017; Zango et al., 2019). This phenomenon is known as individual specialisation (Bolnick et al., 2003). The degree of individual specialisation in a population has important evolutionary and conservation implications. Natural selection may select for (Traisnel and Pichegru,

2019; Zango et al., 2019) or against (Darimont et al., 2007) individual specialisation depending on the environment, and extreme cases of individual specialization can lead towards reproductive isolation and sympatric speciation (Bolnick, 2004). From the conservation point of view, different specialised individuals of one population occupy different niches and may be affected by different environmental impacts (Durell, 2000). Also, individual specialists may not be able to change their behaviour as adults, even when their habitat is sub-optimal (Cardona et al., 2017; Vander Zanden et al., 2016). Thus, the degree of individual specialisation is a necessary information to properly manage a population.

In some species, the individuals of a population are generalist during their youth and become specialists at some point of their development (Cardona et al., 2017; Vander Zanden et al., 2013). Thus, these individuals explore different habitats or behaviours before choosing to the best one. The transition towards specialization would be especially important in long-lived species since they are more likely to encounter a change in the environment that force them to shift their behaviour or habitat. The transition can be detected by analysing metabolically inert tissues that accumulate over a long period of time (Vander Zanden et al., 2016), such as baleen whale earplugs.

Similarly to some bones, otoliths, baleen plates, hair, nails and feathers, the earplug is a biologically inert tissue; thus, it does not change its biogeochemical composition after its formation (Mansouri et al., 2021; Ramos and González-Solís, 2012; Robinson et al., 2013). The earplug is formed inside the auditory canal in baleen whales. The wall of this canal segregate keratin, lipids and cellular debris that accumulate and build up a conus-shaped structure (Roe, 1967). Due to their different lipid content, the pigmentation of the layer deposited in summer is clear while that of the layer deposited in winter is dark (Roe, 1967). The combination of these two layers constitutes a “growth layer group”, which correspond to a year (Laws and Purves, 1956). Chemical tracers, such as stable isotopes, are fixed in these growth layer groups and form a life-long time record. Each layer contains the isotopic information corresponding to the year of life in which the layer group was formed.

Stable isotope ratios have been used in marine mammal tissues to investigate multiple factors such as habitat use, feeding ecology, migration, physiology and nutritive condition, among others (Aguilar et al., 2014; Borrell et al., 2016; Giménez et al., 2013; Rita et al., 2017; Silva et al., 2013; Vighi et al., 2016). In animals that use different habitats or engage in migrations, the variation in stable isotope ratios is analogous to variation in both the isotopic baselines of the locations visited as well as the resources exploited, and provides a means to estimate to what is defined as the isotopic niche (Newsome et al., 2007). An individual would be considered an individual specialist in the isotopic space if the width of its isotopic niche is significantly smaller than the isotopic niche of the population (Bolnick et al., 2003). This means that, despite the range of habitats and resources exploited by the population, this individual consistently exploits

the same subset of habitats and resources over time. The width of the isotopic niche can be estimated as the area of the standard ellipse (SEA) (Jackson et al., 2011), which is an ellipse that encompasses a set percentage of the samples, being 40% a common threshold.

The aim of this study is to determine the degree of individual specialisation, and its consistency over time of a long-lived marine mammal, the fin whale (*Balaenoptera physalus*) using the stable isotopes deposited in its earplugs. The fin whale is a cosmopolite generalist species. During the summer, whales aggregate to feed on krill and schooling fish at high latitude grounds and, during winter, they migrate to the breeding grounds at low latitudes (Aguilar and García-Vernet, 2018). Due to its longevity and wide home range, this species is susceptible to encounter changes in the environment.

Materials and Methods

Sample Collection

The earplugs analysed in this study were collected from 17 fin whales captured during commercial whaling operations off the coast of Galicia (north-west Spain) in 1983 (5), 1984 (10) and 1985 (2) (Table 1). The earplugs were extracted from the whales and preserved in formalin. Only earplugs in which growth layer groups were clearly readable and produced good to excellent age-determinations (Aguilar and Lockyer, 1987) were used for the study. Before conducting the analyses, the earplugs were first rinsed in fresh water for 24 h to eliminate the superficial formalin residuals. Then, they were cut along the longer axis with a razor blade to expose the mid-central section, where the growth layers are more visible. Once this was done, a subsample of each growth layer (comprising both the winter and the summer layers) was extracted using a hypodermic needle.

Table 1. Biological variables and number of samples of the studied individuals.

Whale	Sex	Age at death	# of samples
1983-5	Female	14	11
1983-21	Male	13	10
1983-31	Male	24	21
1983-33	Female	13	10
1983-132	Female	15	12
1984-6	Female	21	18
1984-25	Male	15	12
1984-26	Female	13	10
1984-29	Female	18	15
1984-40	Female	16	13
1984-53	Female	18	15

1984-59	Male	18	15
1984-62	Male	12	9
1984-83	Male	19	16
1984-102	Male	13	10
1985-02	Male	9	6
1985-07	Female	8	5

Stable Isotope Analyses

Samples were dried in an oven at 60ºC and, afterwards, they were left stirring for 24h with chloroform-methanol (2:1), twice, to extract lipids (Bligh and Dyer, 1959). Finally, the delipidized samples were dried again in an oven at 60ºC to eliminate the residual solvent.

Samples (approximately 0.3 mg) and reference materials were weighted into tin cups (3.3 x 5 mm) and analysed for stable isotope ratios using an elemental analyser (model FlashEA 1112, TermoFisher Scientific, Milan, Italy) coupled with a Delta C isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany).

Stable isotope ratios are expressed in delta (δ) with relative variations of stable isotope ratios expressed in per mil (‰) deviations from predefined international standards, and they were calculated as:

$$\delta\ {}^{j}X = \frac{({}^{j}X/\ {}^{i}X)_{sample}}{({}^{j}X/\ {}^{i}X)_{standard}} - 1$$

where ^{j}X is the heavier isotope (^{13}C or ^{15}N) and ^{i}X is the lighter isotope (^{12}C or ^{14}N) in the analytical sample and in the international measurement standard (Bond and Hobson, 2012); international standards were the Vienna Pee Dee Belemnite (VPDB) calcium carbonate for the $\delta^{13}C$ value and atmospheric nitrogen (air) for the $\delta^{15}N$ value. However, data were normalized using commercially available laboratory reference materials. For carbon, isotopic reference materials of known ^{13}C:^{12}C ratios, as given by the International Atomic Energy Agency (IAEA, Vienna, Austria), were used for calibration at a precision of 0.05 ‰. These include polyethylene (IAEA-CH-7, $\delta^{13}C$= –32.1 ‰), L-glutamic acid (IAEA-USGS40, $\delta^{13}C$ = -26.4 ‰), and sucrose (IAEA-CH-6, $\delta^{13}C$= –10.4 ‰). For nitrogen, secondary isotopic reference materials of known ^{15}N:^{14}N ratios, namely $(NH_4)_2SO_4$ (IAEA-N1, $\delta^{15}N$ = +0.4 ‰ and IAEA-N2, $\delta^{15}N$ = +20.3 ‰), L-glutamic acid (IAEA-USGS40, $\delta^{15}N$= -4.5 ‰) and KNO_3 (IAEA-NO3, $\delta^{15}N$= +4.7 ‰), were used to a precision of 0.2 ‰. These isotopic reference materials were used to recalibrate the system and compensate for any measurement of drift over time once every 12 samples tested. The earplugs subsampling and the stable isotope analysis were conducted in the University of Barcelona.

Because the samples had been collected in the early 1980s, for the statistical analysis the $\delta^{13}C$ values were corrected for the Suess effect, which refers to the increment of ^{13}C in the atmosphere due to the burn of fossil fuels. We applied the following correction formula to standardize all the results to the 1985 $\delta^{13}C$ baseline (Borrell et al., 2018).

$$\delta^{13}C_{corr} = \delta^{13}C + 0.016 * (1985 - year)$$

where *year* is the year in which the growth layer group of the sample was formed (year of death minus the number of growth layer groups between the sample and the last growth layer group).

Individual Specialisation Index

The individual specialisation index, using stable isotopes, was calculated as the ratio between the intra-individual isotope ratio variability and that of the population (Bolnick et al., 2002). To avoid the possible effect of lactation (which increases the stable isotope ratios of the calf), the first 3 years of the whales were not included in this analysis. In order to calculate the index using both stable isotopes, the standard ellipse area was used as a proxy of the variability (Jackson et al., 2011). Thus, the intraindividual variability was measured as the standard ellipse area of all the $\delta^{15}N$ and $\delta^{13}C$ values of one individual, and the population variability was measured as the standard ellipse area of the whole $\delta^{15}N$ and $\delta^{13}C$ values data set –all years of every whale in the population.

A bootstrap analysis was performed to assess the probability that the observed pattern of individual specialisation in the population might have emerged by chance. A total of 1000 populations of 20 generalist fin whales were simulated by assigning to each whale 10 $\delta^{13}C$ values and 10 $\delta^{15}N$ values randomly sampled from the original data set. Later, the individual specialisation index was computed for each simulated individual within its population. Only those whales with a lower individual specialisation index than 95% of the simulated generalist whales were considered individual specialists.

In order to measure the temporal consistency of the individual specialisation, the individual specialisation index was also calculated for each individual in three different periods: between 4 and 8 years, between 9 and 13 years and between 14 and 18 years. A bootstrap analysis was also performed for each period to test the statistical significance of the individual specialisation index. For each period, 1000 populations of 20 individuals were simulated by assigning to each whale 5 $\delta^{13}C$ values and 5 $\delta^{15}N$ values of the real population from the same period. As in the previous analysis, the results of this simulated generalist population were used to calculate the threshold under which an individual is identified as an individual specialist.

The T-student test was used to establish the statistical significance of the difference among the two sexes. The variation in the individual specialization index was tested using a generalized linear model (glm). All statistics were performed using the

statistical program R (R Core Team, 2020). The standard ellipse areas were measured using the package SIBER (Jackson et al., 2011) of the statistical program R (R Core Team, 2020).

Results

The average individual specialisation index of the population was 0.39. Most of the individuals (76%) had an individual specialisation index below 0.46 (the threshold calculated with the simulated population) and can be considered individual specialists (Fig 1). Although females appeared to have a lower individual specialisation index than the males (0.35 and 0.43, respectively), the difference was not significant (t = 0.44, d.f. = 1, 15, p-value = 0.66).

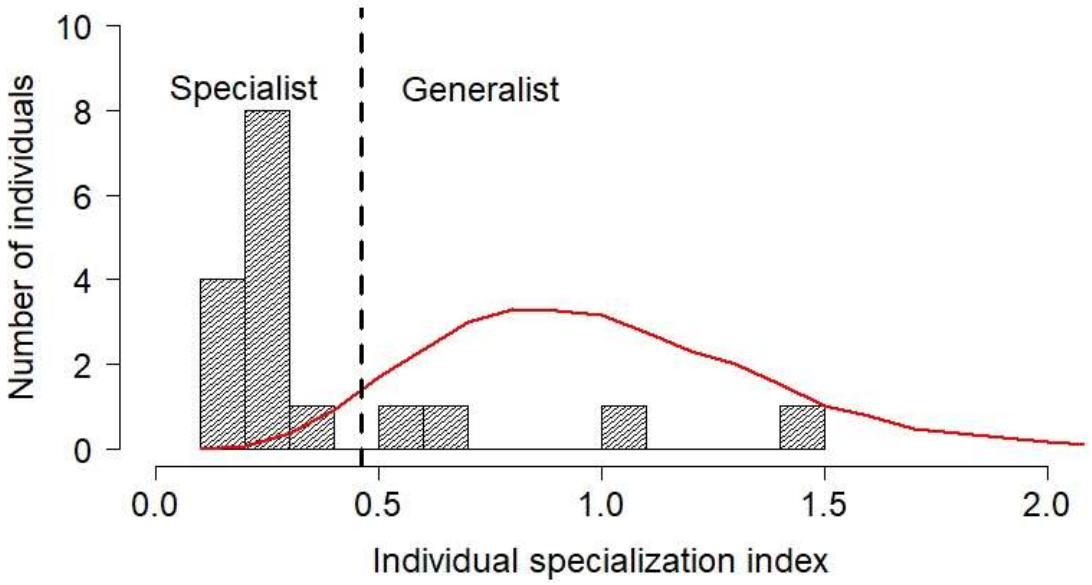

Figure 1. Frequency distribution of the individual specialisation index of the real population (bars) and the expected distribution of a generalist population (red line). The vertical dashed line represents the threshold below which individuals are considered individual specialist.

The average individual specialisation index did not differ among the three periods (F = 1.025, d.f. = 2, 34, p-value = 0.37). Approximately, 65% of individuals were individual specialists during the first period, 64% during the second and 66% during the third (Fig. 2).

Although the proportion of individual specialist whales was similar during the three periods, the whales that were considered individual specialists was different in each period. On several occasions, a whale was considered an individual specialist during a period and a generalist during the next. For example, whale 84006 was generalist during the first period, individual specialist during the second one and generalist again in the third period (Fig. 3 top) and whale 83031 was generalist during the first period,

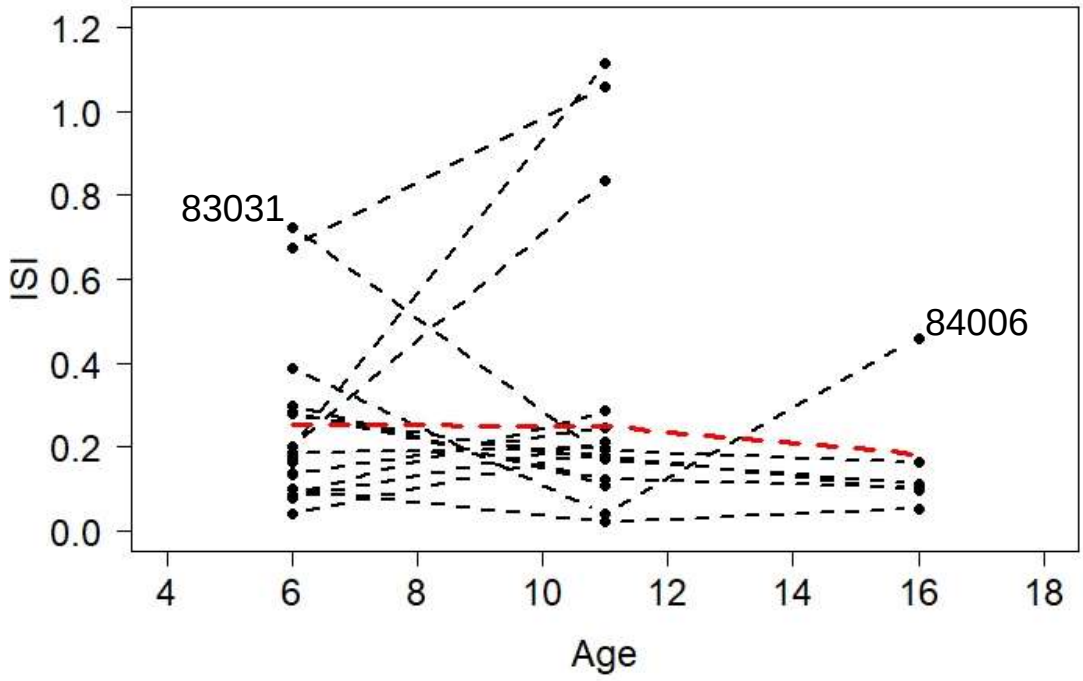

Figure 2. Individual specialisation index (ISI) in the three studied periods. The red dashed line represents the threshold below which individuals are considered individual specialists. Two depicted whales are examples of a whales varying their specialization strategy (see Fig.3).

but it became progressively more individual specialist in the following ones (Fig. 3 bottom).

Discussion

The results showed that the Spanish fin whale population is mainly composed of individual specialists. This strategy seems to be established quite early in their lives since a high percentage of the subadult animals (i.e. under the age of 8) already were individual specialists. The proportion of individual specialists was very similar among the three periods, which suggests that the advantages of the individual specialization may be present during the entire life of the whale.

Individual specialists often have greater fitness than generalists in situations of intense intraspecific competition (Svanbäck and Bolnick, 2007) or in scarcity of prey (Tinker et al., 2008; Traisnel and Pichegru, 2019). This may explain why fin whales have evolved as individual specialists. This species spends the summer in the feeding grounds at high latitudes, where they aggregate and possibly compete with other conspecifics; and they spend the winter in the breeding grounds, were they suffer nutritional stress, possibly due to the scarcity of prey (Aguilar and García-Vernet, 2018). Thus, fin whales are likely to benefit from being individual specialist throughout the year.

In the studied population, the individual specialisation index varied in many of the individuals and, in four occasions, one individual considered individual specialist

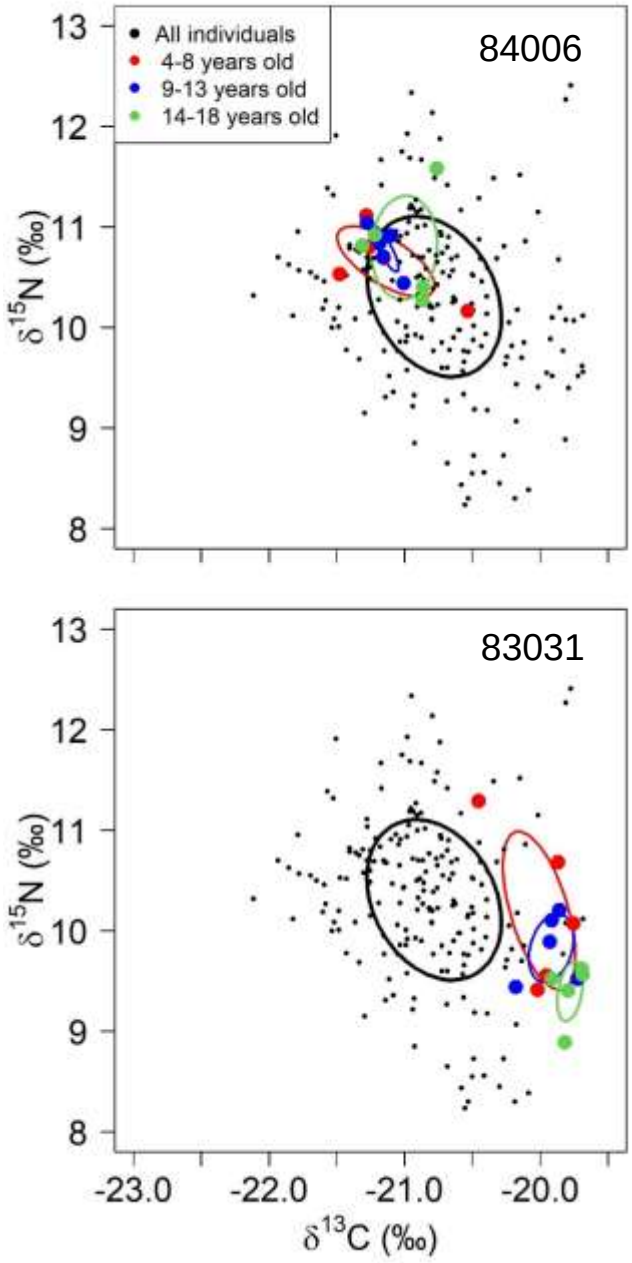

Figure 3. Examples of changing individual specialisation index in two whales. For each whale, the samples of the three study periods and its standard ellipses are shown in red (4-8 years), blue (9-13 years) and green (14-18 years). The whole population, and its standard ellipse is shown in black. 84006 (top) appeared to be generalist during the first period, specialist during the second and generalist again in the third period and whale 83031 (bottom) was generalist during the first period, but it became progressively more specialist in the following ones.

during a period could be considered generalist during the next period (Fig. 2 and Fig. 3). One of the possible causes of this variation is the variability of the environment. For example, the fin whale is often regarded as an opportunistic species, which mainly feed on krill but may also exploit fish in the absence of euphausiids. Thus, a variation in the abundance of krill produce a change in the diet of fin whales and thus the interannual variability in stable isotopes. However, another possible explanation is that fin whale individuals may be able to modify and expand their isotopic niche in certain conditions.

The main disadvantage of the individual specialisation is that the individuals of a population may struggle to adapt to changes in the environment and to colonize new territory (Cardona et al., 2017; Vander Zanden et al., 2013). Individual specialisation may be irreversible when it is based on morphological traits (Svanbäck and Bolnick, 2007), which cannot be modified as an adult, but, when the individual specialisation is caused by extrinsic factors, long-term behaviour shifts to adapt to environmental changes may be expected (Bell et al., 2009; McHuron et al., 2018; Woo et al., 2008). A similar situation could potentially explain why some fin whales expanded their isotopic niche and became generalists for a period of time. If they changed their behaviour when the environmental conditions vary, they could explore and exploit different resources and locations to those they had used in the past, which would help them to offset the disadvantages of being individual specialists.

It is still unclear why the individual specialist behaviour has been developed in fin whales, and why some individuals seem to vary their behaviour at some point of their life. Further research should focus on the evolutionary causes of this behaviour and stablish when it is selected in favour or against. Coping with climate change is hard for most species and may be specially challenging for those with a high proportion of individual specialists. A better understanding on the roots of the individual specialisation in fin whales will improve our inferences of the effect of a changing environment on this species.

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References

Aguilar, A., García-Vernet, R., 2018. Fin Whale (Balaenoptera physalus), in: Würsig, B., Thewissen, J.G.M., Kovacs, K.M. (Eds.), Encyclopedia of Marine Mammals. Elsevier, Amsterdam (Netherland), pp. 368–371. https://doi.org/10.1016/B978-0-12-

804327-1.00128-X

Aguilar, A., Giménez, J., Gómez-Campos, E., Cardona, L., Borrell, A., 2014. $\delta^{15}N$ value does not reflect fasting in mysticetes. PLoS One 9, e92288. https://doi.org/10.1371/journal.pone.0092288

Aguilar, A., Lockyer, C.H., 1987. Growth, physical maturity, and mortality of fin whales (Balaenoptera physalus) inhabiting the temperate waters of the northeast Atlantic. Can. J. Zool. 65, 253–264.

Bell, A.M., Hankison, S.J., Laskowski, K.L., 2009. The repeatability of behaviour: a meta-analysis. Anim. Behav. 77, 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. https://doi.org/dx.doi.org/10,1139/cjm2014-0700

Bogusch, P., Kratochvíl, L., Straka, J., 2006. Generalist cuckoo bees (Hymenoptera: Apoidea: Sphecodes) are species-specialist at the individual level. Behav. Ecol. Sociobiol. 60, 422–429. https://doi.org/10.1007/s00265-006-0182-4

Bolnick, D.I., 2004. Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. Evolution (N. Y). 58, 608–618. https://doi.org/10.1111/j.0014-3820.2004.tb01683.x

Bolnick, D.I., Svanbäck, R., Fordyce, J. a, Yang, L.H., Davis, J.M., Hulsey, C.D., Forister, M.L., 2003. The ecology of individuals: incidence and implications of individual specialization. Am. Nat. 161, 1–28. https://doi.org/10.1086/343878

Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M., Svanbäck, R., 2002. Measuring individual-level resource specialization. Ecology 83, 2936–2941.

Bond, A.L., Hobson, K.A., 2012. Reporting stable-isotope ratios in ecology: recommended terminology, guidelines and best practices. Waterbirds 35, 324–331. https://doi.org/10.1675/063.035.0213

Borrell, A., Gómez-Campos, E., Aguilar, A., 2016. Influence of reproduction on stable isotope ratios: nitrogen and carbon isotope discrimination between mothers, fetuses, and milk in the fin whale, a capital breeder. Physiol. Biochem. Zool. 89, 41–50. https://doi.org/10.1086/684632

Borrell, A., Saiz, L., Víkingsson, G.A., Gaufier, P., López Fernández, A., Aguilar, A., 2018. Fin whales as bioindicators of multi-decadal change in carbon and oxygen stable isotope shifts in the North Atlantic. Mar. Environ. Res. 138, 129–134. https://doi.org/10.1016/j.marenvres.2018.04.014

Cardona, L., Martins, S., Uterga, R., Marco, A., 2017. Individual specialization and behavioral plasticity in a long-lived marine predator. J. Exp. Mar. Bio. Ecol. 497, 127–133. https://doi.org/10.1016/j.jembe.2017.09.021

Darimont, C.T., Paquet, P.C., Reimchen, T.E., 2007. Stable isotopic niche predicts fitness of prey in a wolf-deer system. Biol. J. Linn. Soc. 90, 125–137.

https://doi.org/10.1111/j.1095-8312.2007.00716.x

Durell, S.E.A., 2000. Individual feeding specialisation in shorebirds: Population consequences and conservation implications. Biol. Rev. 75, 503–518. https://doi.org/10.1111/j.1469-185X.2000.tb00053.x

Giménez, J., Gómez-Campos, E., Borrell, A., Cardona, L., Aguilar, A., 2013. Isotopic evidence of limited exchange between Mediterranean and eastern North Atlantic fin whales. Rapid Commun. Mass Spectrom. 27, 1801–1806. https://doi.org/10.1002/rcm.6633

Hoelzel, A.R., Dorsey, E.M., Stern, S.J., 1989. The foraging specializations of individual minke whales. Anim. Behav. 38, 786–794. https://doi.org/10.1016/S0003-3472(89)80111-3

Hückstädt, L.A., Koch, P.L., McDonald, B.I., Goebel, M.E., Crocker, D.E., Costa, D.P., 2012. Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. Oecologia 169, 395–406. https://doi.org/10.1007/s00442-011-2202-y

Jackson, A.L., Parnell, A.C., Inger, R., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 80, 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Laws, R.M., Purves, P.E., 1956. The ear plug of the Mysticeti as an indication of age with special reference to the North Atlantic fin whale (*Balaenoptera physalus* Linn.). Nor. Hvalfangst-Tidende 45, 413–425.

Mansouri, F., Winfield, Z.C., Crain, D.D., Morris, B., Charapata, P., Sabin, R., Potter, C.W., Hering, A.S., Fulton, J., Trumble, S.J., Usenko, S., 2021. Evidence of multi-decadal behavior and ecosystem-level changes revealed by reconstructed lifetime stable isotope profiles of baleen whale earplugs. Sci. Total Environ. 757. https://doi.org/10.1016/j.scitotenv.2020.143985

McHuron, E.A., Hazen, E., Costa, D.P., 2018. Constrained by consistency? Repeatability of foraging behavior at multiple timescales for a generalist marine predator. Mar. Biol. 165, 1–13. https://doi.org/10.1007/s00227-018-3382-3

Mery, F., Burns, J.G., 2010. Behavioural plasticity: An interaction between evolution and experience. Evol. Ecol. 24, 571–583. https://doi.org/10.1007/s10682-009-9336-y

Newsome, S.D., Rio, Martinez del, C., Bearhop, S., Phillips, D.L., 2007. A niche for isotope ecology. Front. Ecol. Environ. 5, 429–436. https://doi.org/10.1890/060150.01

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramos, R., González-Solís, J., 2012. Trace me if you can: The use of intrinsic biogeochemical markers in marine top predators. Front. Ecol. Environ. 10, 258–266. https://doi.org/10.1890/110140

Rita, D., Drago, M., Galimberti, F., Cardona, L., 2017. Temporal consistency of individual

trophic specialization in southern elephant seals Mirounga leonina. Mar. Ecol. Prog. Ser. 585, 229–242. https://doi.org/https://doi.org/10.3354/meps12411

Robinson, E.M., Trumble, S.J., Subedi, B., Sanders, R., Usenko, S., 2013. Selective pressurized liquid extraction of pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in a whale earplug (earwax): A novel method for analyzing organic contaminants in lipid-rich matrices. J. Chromatogr. A 1319, 14–20. https://doi.org/10.1016/j.chroma.2013.10.023

Roe, H.S.J., 1967. Seasonal formation of laminae in the ear of the fin whale. Discov. reports 35, 1–30.

Silva, M.A., Prieto, R., Jonsen, I., Baumgartner, M.F., Santos, R.S., 2013. North Atlantic blue and fin whales suspend their spring migration to forage in middle latitudes: building up energy reserves for the journey? PLoS One 8. https://doi.org/10.1371/journal.pone.0076507

Svanbäck, R., Bolnick, D.I., 2007. Intraspecific competition drives increased resource use diversity within a natural population. Proc. R. Soc. B Biol. Sci. 274, 839–844. https://doi.org/10.1098/rspb.2006.0198

Tinker, M.T., Bentall, G., Estes, J.A., 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proc. Natl. Acad. Sci. U. S. A. 105, 560–565. https://doi.org/10.1073/pnas.0709263105

Traisnel, G., Pichegru, L., 2019. Success comes with consistency in hard times: Foraging repeatability relates to sex and breeding output in African penguins. Mar. Ecol. Prog. Ser. 608, 279–289. https://doi.org/10.3354/meps12827

Trumble, S.J., Norman, S.A., Crain, D.D., Mansouri, F., Winfield, Z.C., Sabin, R., Potter, C.W., Gabriele, C.M., Usenko, S., 2018. Baleen whale cortisol levels reveal a physiological response to 20th century whaling. Nat. Commun. 9. https://doi.org/10.1038/s41467-018-07044-w

Trumble, S.J., Robinson, E.M., Berman-Kowalewski, M., Potter, C.W., Usenko, S., 2013. Blue whale earplug reveals lifetime contaminant exposure and hormone profiles. Proc. Natl. Acad. Sci. U. S. A. 110, 1–5. https://doi.org/10.1073/pnas.1311418110

Vander Zanden, H.B., Bjorndal, K.A., Bolten, A.B., 2013. Temporal consistency and individual specialization in resource use by green turtles in successive life stages. Oecologia 173, 767–777. https://doi.org/10.1007/s00442-013-2655-2

Vander Zanden, H.B., Bolten, A.B., Tucker, A.D., Hart, K.M., Lamont, M.M., Fujisaki, I., Reich, K.J., Addison, D.S., Mansfield, K., Phillips, K.F., Pajuelo, M., Bjorndal, K.A., 2016. Biomarkers reveal sea turtles remained in oiled areas following the Deepwater Horizon oil spill. Ecol. Appl. 26, 2145–2155. https://doi.org/10.1002/eap.1366

Vighi, M., Borrell, A., Aguilar, A., 2016. Stable isotope analysis and fin whale subpopulation structure in the eastern North Atlantic. Mar. Mammal Sci. 32, 535–551. https://doi.org/10.1111/mms.12283

Woo, K.J., Elliott, K.H., Davidson, M., Gaston, A.J., Davoren, G.K., 2008. Individual

specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. J. Anim. Ecol. 77, 1082–1091. https://doi.org/10.1111/j.1365-2656.2008.01429.x

Zango, L., Reyes-González, J.M., Militão, T., Zajková, Z., Álvarez-Alonso, E., Ramos, R., González-Solís, J., 2019. Year-round individual specialization in the feeding ecology of a long-lived seabird. Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-48214-0

Discusión General

Discusión General

A pesar de que el rorcual común es uno de los misticetos más abundantes del mar Atlántico, su comportamiento durante el invierno continúa siendo, en gran parte, un misterio. En otoño, tras haberse alimentado en las zonas de veraneo, la mayoría de los rorcuales comunes migran a zonas más cálidas, donde se reproducen. La localización y el comportamiento del rorcual en estas zonas son desconocidos y por eso el objetivo general de esta tesis es la de mejorar nuestra comprensión de estas migraciones en las poblaciones del Atlántico Norte. En cada capítulo de la tesis se ha analizado un marcador químico en un tejido diferente: grasa y contenido estomacal, barba de ballena y conos de cera. Esto ha proporcionado información sobre el hábitat y la dieta del rorcual común en tres escalas temporales diferentes. Los resultados han sido puestos en contexto en sendas discusiones y, en este apartado, se resume la información más relevante de cada capítulo, y se combina para generar un marco general de las migraciones del rorcual común del Atlántico Norte.

En el primer capítulo se quiso obtener más información sobre el hábitat del rorcual común en una escala temporal corta y media. Para ello, se desarrolló un método de detección de alquenonas en tejidos de ballena como la grasa hipodérmica de ballena y el contenido estomacal. El contenido estomacal proporcionó información sobre la temperatura del agua pocas semanas antes de la captura del animal, mientras que la grasa hipodérmica proporcionó información de las alquenonas ingeridas durante un período que nos eludo determinar al desconocerse la tasa de recambio de las alquenonas en este tejido. Se comprobó que las alquenonas no se bioacumulan en la grasa y que su tasa de recambio es, como mínimo, mayor que el período durante el que se obtuvieron las muestras: unos tres meses. Por ello, es muy probable que la información contenida en este tejido sea un promedio entre las zonas de alimentación y las zonas de invernada.

El índice $U^{k'}_{37}$ calculado en las muestras de contenido estomacal refleja la temperatura ambiental de las zonas de veraneo. Se pudo observar la correlación entre el índice $U^{k'}_{37}$ y la temperatura ambiental local en el noroeste de la Península Ibérica y, también, la diferencia de temperatura entre las dos áreas estudiadas. En cambio, el índice $U^{k'}_{37}$ medido en la grasa hipodérmica de ballena no mostró un cambio a lo largo del tiempo de recolección de las muestras. Además, fue más variable que en las muestras de contenido estomacal.

En la primera parte del segundo capítulo se quiso determinar cómo se sintetiza la barba de ballena. Para ello, se realizaron cortes histológicos en la base de la barba y se observó que la parte exterior de la barba, la corteza, el tejido se forma antes y más rápidamente que en la parte interior, la médula. Esto llevó a la conclusión que los futuros análisis de isótopos estables en barba de ballena deberían analizar una única capa de tejido, preferentemente la corteza.

En la segunda parte, el objetivo del estudio consistió en determinar el nivel trófico del rorcual común en invierno y la fuente de nitrógeno en el hábitat donde invernan. Para ello, se analizaron los isótopos del nitrógeno de los aminoácidos específicos (CSIA-AA) en muestras de barba sintetizada en invierno y en verano. Los CSIA-AA, a diferencia de los análisis totales de isótopos de proteínas, permiten determinar la señal ambiental de los isótopos del nitrógeno a la vez que el nivel trófico del individuo. Los resultados mostraron que, durante el invierno, parte de los rorcuales comunes del stock islandés visitan y se alimentan en zonas de emergencia de agua profunda. También pudo observarse que el nivel trófico de los rorcuales comunes islandeses aumenta durante el invierno. Esto podría deberse tanto a un cambio de dieta, es decir a que los rorcuales complementaran su dieta con pescado, o a que el krill incrementara su nivel trófico. Ambas explicaciones son probables y podrían darse simultáneamente.

Finalmente, en el tercer capítulo se quería establecer la consistencia del comportamiento del rorcual común del noroeste español en una escala temporal larga. En este contexto, el comportamiento del rorcual común no se refiere a las áreas de invernada o a la dieta durante el invierno, sino a todas las acciones y decisiones tomadas por el individuo durante un año, integradas en un único valor isotópico. Esta consistencia temporal se midió en los isótopos estables de carbono y nitrógeno en muestras de conos de cera. Los resultados mostraron que, si bien la población de rorcual común puede considerarse generalista por la cantidad de estrategias que presenta, los individuos de esta población son especialistas individuales, es decir, repiten comportamientos similares cada año. También se observó que en ciertas situaciones algunos individuos especialistas se volvían generalistas durante un periodo de tiempo. La razón de esta reversión es desconocida; podría deberse a variaciones ambientales en las zonas donde el individuo se alimentó, o a un cambio en el comportamiento del animal, ya sea de la zona visitada en verano o en invierno o de la dieta. Si la segunda hipótesis fuera la correcta, eso implicaría que los rorcuales comunes son capaces de revertir su especialización individual y modificar temporalmente su estrategia en ciertas condiciones.

En conjunto:

Las poblaciones son más generalistas…

Los datos obtenidos en la presente tesis muestran que, en general, los dos stocks de rorcual común estudiados se vuelven más generalistas durante el invierno que durante el verano, tanto en su dieta como en su hábitat.

Por un lado, las alquenonas detectadas en muestras de grasa hipodérmica, ingeridas probablemente a lo largo del año, muestran una variabilidad en la temperatura estimada que no puede ser explicada por el cambio de temperatura del verano, a diferencia de las muestras de contenido estomacal. Esto sugiere que las alquenonas presentes en la grasa hipodérmica son una mezcla de las alquenonas ingeridas a lo largo de un período amplio, que seguramente incluye la época invernal y estival. Por tanto, la variabilidad en el índice $U^{k'}_{37}$ en la grasa de ballena seguramente esté relacionado con la variabilidad en la temperatura del agua de las zonas visitadas por las diferentes ballenas. También los análisis de isótopos del nitrógeno en aminoácidos (CSIA-AA) muestran una mayor variabilidad durante la estación invernal, sugiriendo, en este caso, que los rorcuales comunes tienen una dieta más generalista durante el invierno. El nivel trófico detectado en estos rorcuales es más alto durante el invierno que durante el verano. Este cambio de nivel trófico no puede atribuirse definitivamente a un cambio de dieta, ya que el krill también cambia su nivel trófico durante el invierno. Sin embargo, la mayor variabilidad en el nivel trófico de los rorcuales en invierno sugiere que algunos individuos podrían complementar su dieta con pescado. Esto les permitiría obtener alimento de manera oportunista en ausencia de krill, pero también aprovechar las agregaciones estacionales predecibles que hacen estas presas durante su ciclo anual, como sucede en la costa irlandesa donde la presencia de rorcuales coincide con la época de desove del espadín (*Sprattus sprattus*) y el arenque (*Clupea harengus*) (Whooley et al. 2011)

Los resultados obtenidos aquí son compatibles con aquellos obtenidos en otros stocks de la misma especie. Por ejemplo, los estudios de telemetría muestran que los rorcuales comunes de una misma zona de veraneo migran a zonas diferentes durante el otoño, tanto en los stocks atlánticos como en otras poblaciones (Bentaleb et al. 2011; Lydersen et al. 2020). Además, Los estudios acústicos también muestran que durante el invierno los rorcuales comunes ocupan grandes porciones de océano, pudiendo ser detectados tanto a lo largo de la dorsal atlántica como en zonas cercanas a la plataforma continental (Nieukirk et al. 2012; Pereira et al. 2020; Romagosa et al. 2020). Se ha propuesto que, debido a la disminución en la producción primaria durante el invierno, los rorcuales comunes se vuelven generalistas, dispersándose por el mar abierto pero manteniéndose siempre a distancia acústica (Notarbartolo di Sciara et al. 2003). Esta distancia puede ser del orden de 100 km, dependiendo de la profundidad en la que se encuentre el animal, el ruido y el fondo marino (Stafford et al. 2007). Esto les permitiría

alimentarse durante el invierno pero continuar comunicándose, lo que parece ser vital durante la época reproductiva (Croll et al. 2002).

…pero generalista no significa aleatorio

Aunque las poblaciones de rorcual común parece que se dispersan durante el invierno y se vuelven más generalistas, esto no significa que los individuos no seleccionen el hábitat donde invernarán.

Los resultados de CSIA-AA muestran que los rorcuales comunes de Islandia pasan el invierno en zonas con emergencia de agua profunda. En el mar abierto, la materia orgánica tiende a precipitar, llevándose consigo los nutrientes y elementos esenciales para la vida. Cuando estos elementos han sido utilizados o han precipitado, los productores primarios no pueden realizar la fotosíntesis, lo que limita los recursos disponibles para los consumidores secundarios. En las condiciones adecuadas, como en zonas de emergencia de aguas costeras o vórtices fríos creados por corrientes, el agua profunda, rica en nutrientes, puede emerger a las capas superficiales. En estas zonas se produce una estimulación de la producción primaria (Martin and Richards 2001; McGillicuddy et al. 2003; Bode et al. 2020) que atrae consumidores secundarios (Godø et al. 2012). Estos "oasis en el mar" son también aprovechados por varias especies de mamíferos marinos, incluyendo los rorcuales comunes (Bailleul et al. 2010; Garcia-Rojas et al. 2018; Pérez-Jorge et al. 2020). A diferencia de los estudios de telemetría, que tan solo relacionan la presencia de rorcual común con zonas de emergencia de agua profunda, los resultados presentados aquí confirman que estas zonas son las principales fuentes de alimentación de los rorcuales durante el invierno. No se descarta que los rorcuales puedan visitar o utilizar también zonas oligotróficas del océano, pero la alimentación en estas sería reducida ya que no dejan una huella isotópica apreciable.

Además de seleccionar zonas específicas para pasar el invierno, parece que los rorcuales comunes tienden a migrar a las mismas zonas cada año. Si bien la población de rorcual común se vuelve más generalista durante el invierno, cada individuo tiende a repetir el mismo comportamiento (zonas visitadas, dieta consumida) cada año. Esta característica, en la que el individuo ocupa una porción pequeña del nicho poblacional disponible, se conoce como especialización individual (Bolnick et al. 2003). En el caso de la población estudiada, la especialización individual es moderada y, interesantemente, reversible en algunos casos.

La especialización individual es una característica que se está detectando cada vez en más especies. A nivel evolutivo, la especialización individual tiende a seleccionarse en aquellas situaciones en que los recursos son escasos y/o predecibles (Araújo et al. 2011; Dermond et al. 2018). Por ejemplo, se podría considerar que los misticetos son especialistas individuales durante el verano, ya que, teniendo disponibles múltiples zonas de alimentación, tienden a visitar siempre la misma. Esta especialización está seguramente causada por la presencia de recursos predecibles en estas áreas

durante el verano. Sin embargo, durante el invierno, los individuos de un mismo stock de alimentación explotan recursos diferentes de manera consistente. No se sabe por qué los individuos migran a zonas diferentes, pero podría deberse a cualquiera de las dos opciones mencionadas anteriormente: la escasez de recursos, pues los individuos especialistas son más eficientes capturando sus presas, o la predictibilidad de los recursos en las zonas de emergencia de agua profunda.

También se desconocen las razones próximas que causan esta especialización individual. En general, la especialización individual puede deberse a razones intrínsecas –directamente relacionadas con la fisiología o morfología del individuo (Svanbäck and Bolnick 2007; Maldonado et al. 2019)– o extrínsecas –relacionadas con el ambiente o la experiencia del individuo (Vander Zanden et al. 2013; Cardona et al. 2017). En otras especies de misticeto, parece que la localización de las zonas de alimentación e invernada son conocimientos transmitidos culturalmente. Las crías nacen en las zonas de invernada y realizan una migración a las zonas de veraneo siguiendo a la madre (Carroll et al. 2015; Richard et al. 2018); por tanto, en estas especies la especialización individual estaría causada por razones extrínsecas. Es posible que también éste sea el caso del rorcual común. Por ejemplo, la población del estrecho de Gibraltar, una de las más intensamente explotadas a principios del siglo XX (Sanpera and Aguilar 1992), desapareció debido a la caza de ballenas y no se ha recuperado (Clapham et al. 2008). Este hecho es sorprendente dada la recuperación que han sufrido otras poblaciones del Atlántico Norte (Vikingsson et al. 2009) y al paso frecuente de ballenas por el lugar (Gauffier et al. 2018; Pereira et al. 2020). Se ha propuesto que, en el caso de que la localización de las zonas de veraneo se transmitiera culturalmente, esta información podría haberse perdido cuando se erradicó la población que habitaba el lugar (Clapham et al. 2008).

Finalmente, cabe mencionar la variación en el índice de especialización individual en la población de rorcual común. Esta variación podría haber sido producida por un cambio ambiental o por un cambio en el comportamiento de los animales. Si la segunda opción fuera la correcta supondría que los individuos están cambiando su estrategia de especialista individual a generalista. La capacidad de revertir la especialización individual ha sido descrita en otras especies, donde individuos especialistas cambiaban su comportamiento a largo plazo para adaptarse a cambios ambientales (Woo et al. 2008; Bell et al. 2009; McHuron et al. 2018). Esta capacidad, sin embargo, no está muy extendida y no puede producirse si la especialización individual está causada por razones intrínsecas o si el animal no puede aprender nuevos comportamientos pasada una cierta edad (Vander Zanden et al. 2016). En el caso de los rorcuales comunes, un mayor número de muestras es necesario para confirmar que son los individuos los que cambian el comportamiento y no es el ecosistema el que experimenta una variación interanual. Estos resultados son importantes para poder predecir la capacidad de los individuos de adaptarse a cambios en el hábitat, como los que produce el cambio climático.

Comparación con otros misticetos

El mayor problema para comparar los resultados obtenidos en esta tesis con los de estudios realizados en otras especies de misticetos es que las especies mejor estudiadas tienen un comportamiento migratorio marcadamente diferente y las especies más similares han sido pobremente estudiadas.

Las ballenas francas (*Eubalaena spp.*) y yubarta (*Megaptera novaeangliae*), que son las especies cuyo comportamiento invernal ha sido mejor estudiado, pasan los inviernos en zonas cálidas cercanas a la costa (Clapham 2017; Kenney 2017). Durante estos períodos se producen las cópulas y nacen las crías, y prácticamente no se alimentan. La migración a la zona de invernada está probablemente motivada por un conjunto de factores que irían dese el hecho de que las crías necesitan una temperatura de agua elevada para crecer eficientemente, para protegerlas de la depredación de las orcas o por motivos de eficiencia metabólica (Corkeron and Connor 1999). Este modelo se suele a extrapolar a las otras especies de misticetos, aunque en la última década se ha comprobado que no todas las especies o poblaciones se ajustan a estos patrones (Geijer et al. 2016).

En general, los balenópteros son especies más oceánicas que las especies mencionadas anteriormente, especialmente durante el invierno (Víkingsson and Heide-Jørgensen 2015; Lesage et al. 2017). Sus zonas de invernada son más difusas, y a menudo están relacionadas con características oceánicas que incrementan la producción primaria, como, por ejemplo, zonas de emergencia de agua profunda, torbellinos que forman las corrientes oceánicas o montañas submarinas (Nasu 1966; Mate et al. 1999; Skov et al. 2008; Baines and Reichelt 2014). Este hecho, combinado con el comportamiento revelado por las marcas satelitales y las variaciones de los isótopos estables, parece indicar que los balenópteros sí que se alimentan durante el invierno (Lockyer 1981; Aguilar et al. 2014; Baines and Reichelt 2014; Lesage et al. 2017; Silva et al. 2019). Además de alimentarse durante el invierno, también se alimentan durante las migraciones. Varias especies, como el rorcual azul (*Balaenoptera musculus*) o el rorcual boreal (*Balaenoptera borealis*) parecen utilizar frentes oceánicos, zonas de alta producción primaria formados por el choque de dos masas de agua de temperatura diferente, para alimentarse temporalmente cuando están en tránsito (Etnoyer et al. 2004; Silva et al. 2013). Al igual que el stock analizado en esta tesis, este comportamiento parece ser compartido por los rorcuales comunes del Mar Mediterráneo (Canese et al. 2006; Panigada et al. 2017).

Al igual que en el rorcual común, la dieta del resto de rorcuales ha sido mejor estudiada durante la época estival que durante la invernal. Durante el verano, el rorcual azul, al igual que el común, suele alimentarse de krill (Sears and Perrin 2017), mientras que el rorcual boreal se encuentra en un nivel trófico inferior y se alimenta de copépodos (Horwood 2017). Por otro lado, el rorcual aliblanco (*Balaenoptera acutorostrata*) varía su dieta según la zona y puede alimentase de peces, krill y

copépodos (Perrin et al. 2017). En Islandia, donde las cuatro especies coinciden durante el verano, todas las especies se alimentan, en parte, de krill. La inclusión de otras especies a la dieta (lanzón en la dieta del rorcual aliblanco y copépodos en la del rorcual boreal) y la separación espacio-temporal de las especies permite una segregación del nicho isotópico de los rorcuales (García-Vernet, datos no publicados). Sin embargo, la información sobre la dieta invernal de estas especies es escasa. Los pocos registros de rorcuales alimentándose en invierno sugieren que en el Mediterráneo el rorcual común mantiene una dieta basada en el krill (Canese et al. 2006), mientras que en el Atlántico Norte la dieta de esta especie puede cambiar geográfica y estacionalmente, priorizando el krill cuando éste está disponible (Jonsgård 1966; Kawamura 1980).

La consistencia temporal en el comportamiento invernal es otro campo extremadamente difícil de estudiar en el grupo de los rorcuales. El rorcual común tiene conos de cera relativamente fáciles de muestrear y adecuados para leer la edad del animal, pero ese no es el caso en los otros balenópteros (Aguilar, comunicación personal). Sin los conos de cera, estudiar este aspecto sólo sería posible tomando biopsias o colocando marcas satelitales al mismo individuo múltiples veces en diferentes años, algo extremadamente dificultoso, sino imposible.

La localización geográfica de las áreas de invernada de los rorcuales solo puede averiguarse con seguridad mediante el uso de marcas satelitales. Sin embargo, la dificultad de marcar estas especies junto a la corta duración de fijación que en los rorcuales presentan estas marcas hace que esta técnica sea extremadamente costosa. Cada uno de los estudios realizados informa sobre el comportamiento de unos pocos individuos de una población concreta, pero cada nuevo estudio aporta una pincelada y revela parte de la distribución geográfica de estas especies. Los estudios de marcadores químicos, por otro lado, pueden proporcionar gran cantidad de información sobre las características de los hábitats y la dieta de los individuos. Usando el tejido adecuado, se puede adaptar el estudio a la escala temporal requerida y resolver algunas de las muchas incógnitas que todavía existen en estas especies.

Recapitulación

En resumen, la presente tesis refuerza los trabajos anteriores que muestran que los rorcuales comunes siguen un patrón migratorio particular. Los rorcuales comunes del Atlántico Norte realizan una migración incompleta (no todos los individuos migran) y diferencial (las migraciones se realizan a diferentes zonas). Esto hace que los individuos de una misma población ocupen un área mayor en invierno que en verano, lo que reduce la competencia interespecífica y les permite alimentarse más eficazmente durante el invierno. Al igual que con las zonas de veraneo, cada individuo parece preferir ciertas zonas de invernada. Estas zonas de invernada suelen incluir áreas de emergencia de agua profunda, donde estos individuos se alimentan. Si bien el comportamiento suele ser consistente cada año, algunos individuos pueden cambiar su estrategia, lo que les puede resultar extremadamente útil para sobrevivir en un ambiente cambiante.

Los marcadores químicos usados y desarrollados en esta tesis han demostrado ser excelentes herramientas para estudiar las migraciones de los rorcuales en general. A diferencia de las marcas satelitales o los estudios de acústica, los marcadores químicos no permiten determinar una localización geográfica precisa, pero sí obtener información oceanográfica, como la fuente de nitrógeno o la temperatura, y de dieta del organismo estudiado. Además, el relativo bajo coste de los marcadores químicos permite realizar muestreos amplios y detectar diferencias en los patrones migratorios entre los grupos de edad o de condición reproductora. Si bien este tipo de análisis ha sido ignorado en la presente tesis, que se ha centrado en proporcionar un marco de migración a nivel poblacional, futuros estudios deberían centrarse en detectar estas diferencias intra-poblacionales en la migración.

Conclusiones

Conclusiones

Capítulo 1

-Las alquenonas pueden ser detectadas en el contenido estomacal (krill) de los rorcuales comunes y la grasa hipodérmica, especialmente en las capas internas de ésta. Las alquenonas no se bioacumulan en la grasa hipodérmica y parece haber cierta biodilución en la transferencia otra lo largo de la cadena alimentaria.

-El valor del índice $U^{k'}_{37}$ medido en el contenido en el contenido estomacal refleja la temperatura del agua unas semanas antes del muestreo. El índice $U^{k'}_{37}$ medido en la grasa hipodérmica, por otro lado, parece promediar la temperatura de las zonas de invernada y las zonas de veraneo.

-La variabilidad presente en el índice $U^{k'}_{37}$ en la grasa hipodérmica es un reflejo de la variabilidad en las migraciones de los stocks estudiados, y es consistente con el hecho de que algunas ballenas quizás no migren y permanezcan cerca de las zonas de alimentación durante todo el año.

Capítulo 2

-El estudio histológico e isotópico de las barbas reveló que el tejido de la corteza se sintetiza antes y durante un período más corto que el tejido de la médula. Por ello, es recomendable muestrear únicamente una de las capas de la barba durante los estudios isotópicos, a ser posible la corteza, y este muestreo debería iniciarse en la zona situada dentro de la encía, en un punto lo más cercano al hueso posible.

-El nivel trófico de los rorcuales comunes se torna más variable durante el invierno y aumenta, en promedio, 0.63 puntos con respecto al verano. Esto sugiere que su dieta es más variable y posiblemente consista en una mezcla de krill y peces.

-Los valores isotópicos del nitrógeno basal indican que las zonas donde los rorcuales se alimentan en invierno se encuentran en zonas de emergencia de agua profunda.

Capítulo 3

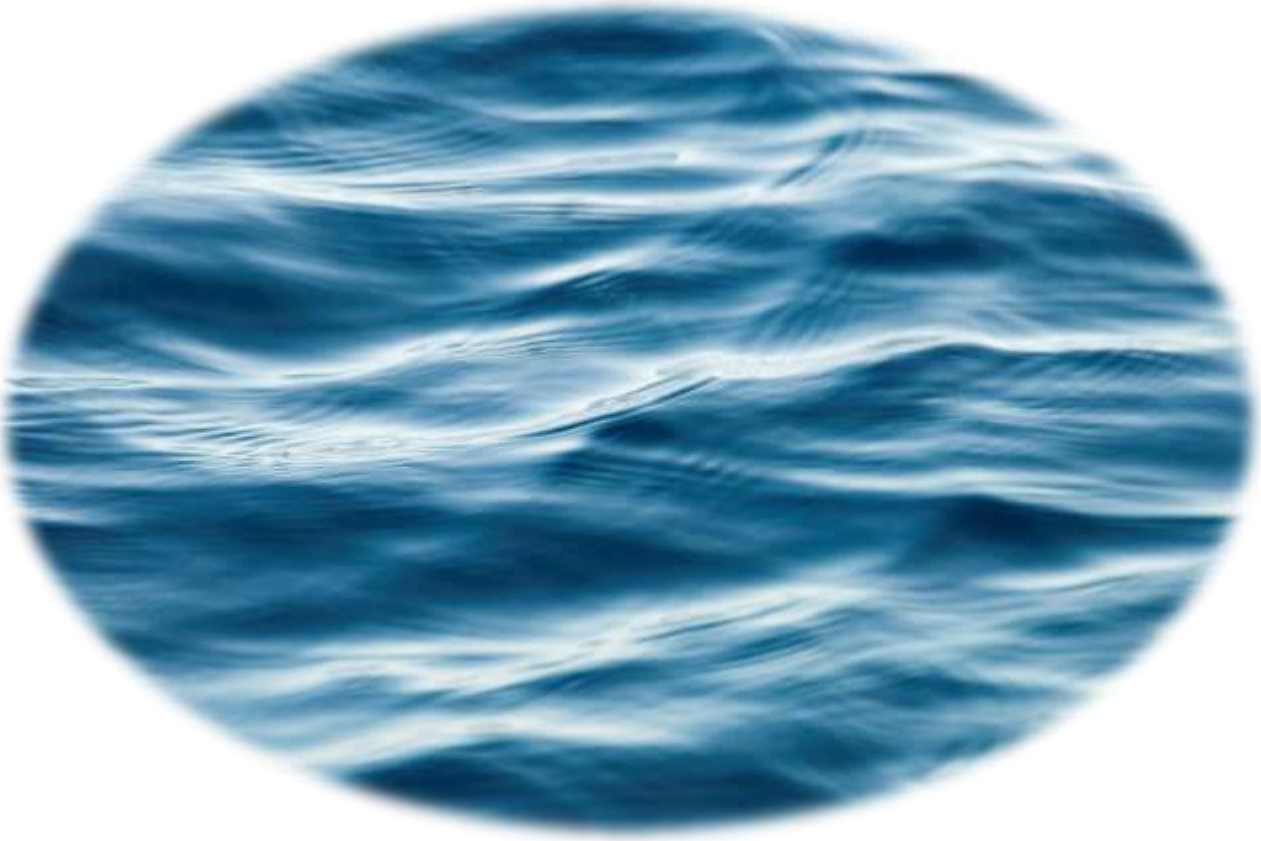
-Más de tres cuartas partes de los rorcuales analizados mostraron un cierto grado de especialización individual, es decir que explotan una parte limitada de todo el nicho isotópico disponible para la población. Las razones de esta especialización individual son

desconocidas, pero podrían deberse a la escasez de recursos durante el invierno i/o a la predictibilidad de las zonas de emergencia de agua profunda.

-En algunos individuos, los valores de especialización individual variaron a lo largo de su vida, lo que podría ser debido a variaciones ambientales, pero también a cambios en el comportamiento.

En conjunto

-En conjunto estos estudios muestran que las poblaciones de rorcual común son más generalistas durante el invierno que durante el verano. Complementan su dieta basada en krill con pescado y encuentran sus presas en zonas de emergencia de agua profunda. Por otro lado, su comportamiento no es aleatorio y parecen seleccionar las zonas de mayor producción primaria durante el invierno. Además, su migración, tanto en lo que se refiere a las zonas visitadas como a la dieta, parece repetirse cada año, pero variar entre los individuos de un mismo stock.



Bibliografía General

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Aguilar, A. 1985. Biología y dinámica poblacional del rorcual común (*Balaenoptera physalus*) en las aguas atlánticas ibéricas. Universitat de Barcelona.

Aguilar, A., and A. Borrell. 1994. Reproductive transfer and variation of body load of organochlorine pollutants with age in fin whales (*Balaenoptera physalus*). Arch. Environmetnal Contam. Toxicol. 27: 546–554.

Aguilar, A., and R. García-Vernet. 2018. Fin Whale (*Balaenoptera physalus*), p. 368–371. In B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.], Encyclopedia of Marine Mammals. Elsevier.

Aguilar, A., J. Giménez, E. Gómez-Campos, L. Cardona, and A. Borrell. 2014. δ15N value does not reflect fasting in mysticetes. PLoS One 9: e92288.

Aguilar, A., and C. H. Lockyer. 1987. Growth, physical maturity, and mortality of fin whales (*Balaenoptera physalus*) inhabiting the temperate waters of the northeast Atlantic. Can. J. Zool. 65: 253–264.

Aguilar, A., M. Olmos, and C. H. Lockyer. 1988. Sexual maturity in fin whales (*Balaenoptera physalus*) caught off Spain. Rep. - Int. Whal. Comm. 38: 317–322.

Araújo, M. S., D. I. Bolnick, and C. A. Layman. 2011. The ecological causes of individual specialisation. Ecol. Lett. 14: 948–958.

Árnason, A. 1995. Genetic markers and whale stocks in the North Atlantic Ocean: a review. Whales, seals, fish man 4: 91–103.

Ayliffe, L. K., T. E. Cerling, T. Robinson, and others. 2004. Turnover of carbon isotopes in tail hair and breath CO2 of horses fed an isotopically varied diet. Oecologia 139: 11–22.

Bailleul, F., C. Cotté, and C. Guinet. 2010. Mesoscale eddies as foraging area of a deep-diving predator, the southern elephant seal. Mar. Ecol. Prog. Ser. 408: 251–264.

Baines, M. E., and M. Reichelt. 2014. Upwellings, canyons and whales: An important winter habitat for balaenopterid whales off Mauritania, northwest Africa. J. Cetacean Res. Manag. 14: 57–67.

Baines, M., M. Reichelt, and D. Griffin. 2017. An autumn aggregation of fin (*Balaenoptera physalus*) and blue whales (*B. musculus*) in the Porcupine Seabight, southwest of Ireland. Deep. Res. Part II Top. Stud. Oceanogr. 141: 168–177.

Baker, C. S., D. Steel, J. Calambokidis, and others. 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Mar. Ecol. Prog. Ser. 494: 291–306.

Bell, A. M., S. J. Hankison, and K. L. Laskowski. 2009. The repeatability of behaviour: a meta-analysis. Anim. Behav. 77: 771–783.

Bendle, J. A. P., and A. Rosell-Melé. 2007. High-resolution alkenone sea surface temperature variability on the North Icelandic Shelf: implications for Nordic Seas palaeoclimatic development during the Holocene. The Holocene 17: 9–24.

Bentaleb, I., C. Martin, M. Vrac, B. Mate, P. Mayzaud, D. Siret, R. De Stephanis, and C. Guinet. 2011. Foraging ecology of Mediterranean fin whales in a changing environment elucidated by satellite tracking and baleen plate stable isotopes. Mar. Ecol. Prog. Ser. 438: 285–302.

Bode, A., A. Lamas, and C. Mompeán. 2020. Effects of upwelling intensity on nitrogen and carbon fluxes through the planktonic food web off A Coruña (Galicia, NW Spain) assessed with stable isotopes. Diversity 12: 1–14.

Bolnick, D. I., R. Svanbäck, J. a Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. Am. Nat. 161: 1–28.

Bond, A. L., and K. A. Hobson. 2012. Reporting stable-isotope ratios in ecology: recommended terminology, guidelines and best practices. Waterbirds 35: 324–331.

Borrell, A., E. Gómez-Campos, and A. Aguilar. 2016. Influence of reproduction on stable isotope ratios: nitrogen and carbon isotope discrimination between mothers, fetuses, and milk in the fin whale, a capital breeder. Physiol. Biochem. Zool. 89: 41–50.

Brassell, S. C., M. Dumitrescu, T. J. Bralower, and others. 2004. Recognition of alkenones in a lower *Aptian porcellanite* from the west-central Pacific. Org. Geochem. 35: 181–188.

Brault, E. K., P. L. Koch, D. P. Costa, and others. 2019. Trophic position and foraging ecology of Ross, Weddell, and crabeater seals revealed by compound-specific isotope analysis. Mar. Ecol. Prog. Ser. 611: 1–18.

Canese, S., A. Cardinali, C. M. Fortuna, M. Giusti, G. Lauriano, E. Salvati, and S. Greco. 2006. The first identified winter feeding ground of fin whales (*Balaenoptera physalus*) in the Mediterranean Sea. J. Mar. Biol. Assoc. United Kingdom 86: 903–907.

Cardona, L., S. Martins, R. Uterga, and A. Marco. 2017. Individual specialization and behavioral plasticity in a long-lived marine predator. J. Exp. Mar. Bio. Ecol. 497: 127–133.

Carroll, E. L., C. S. Baker, M. Watson, and others. 2015. Cultural traditions across a migratory network shape the genetic structure of southern right whales around Australia and New Zealand. Sci. Rep. 5

Castellote, M., C. W. Clark, and M. O. Lammers. 2012. Fin whale (Balaenoptera physalus) population identity in the western Mediterranean Sea. Mar. Mammal Sci. 28: 325–344.

Caut, S. p., S. Laran, E. Garcia-Hartmann, and K. Das. 2011. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). J. Exp. Biol. 214: 538–545.

Charif, R. a, and C. W. Clark. 2009. Acoustic monitoring of large whales in deep waters north and west of the British Isles: 1996-2005. Cornell Lab Ornithol. 08: 40.

Chikaraishi, Y., N. O. Ogawa, Y. Kashiyama, and others. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol. Oceanogr. Methods 7: 740–750.

Christensen, I., T. Haug, and N. Oien. 1992. A review of feeding and reproduction in large baleen whales (Mysticeti) and sperm whales *Physeter macrocephalus* in Norwegian and adjacent waters. Fauna Nor. Ser. A 13: 39–48.

Clapham, P. J. 2017. Humpback whale (Megaptera novaeangliae), p. 489–492. In B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.], Encyclopedia of Marine Mammals. Elsevier.

Clapham, P. J., A. Aguilar, and L. T. Hatch. 2008. Determining spatial and temporal scales for management: Lessons from whaling. Mar. Mammal Sci. 24: 183–201.

Collister, J. W., G. Rieley, B. Stern, G. Eglinton, and B. Fry. 1994. Compound-specific $\delta^{13}C$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms. Org. Geochem. 21: 619–627.

Conte, M. H., M.-A. Sicre, C. Rühlemann, J. C. Weber, S. Schulte, D. Schulz-Bull, and T. Blanz. 2006. Global temperature calibration of the alkenone unsaturation index (Uk'37) in surface waters and comparison with surface sediments. Geochemistry Geophys. Geosystems 7.

Cooke, J. G. 2018. *Balaenoptera physalus*. IUCN Red List Threat. Species 2018 eT2478A50349982.

Corkeron, P. J., and R. C. Connor. 1999. Why do baleen whales migrate. Mar. Mammal Sci. 15: 1228–1245.

Costa, I. R., J. D. Thompson, J. M. Ortega, and F. Prosdocimi. 2014. Metazoan remaining genes for essential amino acid biosynthesis: Sequence conservation and evolutionary analyses. Nutrients 7: 1–16.

Croll, D. a, C. W. Clark, A. Acevedo-Gutiérrez, B. Tershy, S. Flores, J. Gedamke, and J. Urban. 2002. Only male fin whales sing loud songs. Nature 417: 809.

Delarue, J., S. K. Todd, S. M. Van Parijs, and L. Di Iorio. 2009. Geographic variation in Northwest Atlantic fin whale (*Balaenoptera physalus*) song: implications for stock structure assessment. J. Acoust. Soc. Am. 125: 1774–82.

Dermond, P., S. M. Thomas, and J. Brodersen. 2018. Environmental stability increases relative individual specialisation across populations of an aquatic top predator. Oikos 127: 297–305.

Etnoyer, P., D. Canny, B. Mate, and L. Morgan. 2004. Persistent pelagic habitats in the Baja California to Bering Sea (B2B) ecoregion. Oceanography 17: 90–101.

Forcada, J., A. Aguilar, P. Hammond, and X. Pastor. 1996. Distribution and abundance of fin whales (*Balaenoptera physalus*) in the western Mediterranean sea during the summer. J. Zool. 238: 23–34.

Fudge, D. S., L. J. Szewciw, and A. N. Schwalb. 2009. Morphology and development of blue whale baleen: An annotated translation of Tycho Tullberg's classic 1883 paper. Aquat. Mamm. 35: 226–252.

Galligan, T. M., L. H. Schwacke, W. E. Mcfee, and A. S. P. Boggs. 2018. Evidence for cortisol–cortisone metabolism by marine mammal blubber. Mar. Biol. 165: 1–8.

Garcia-Garin, O., M. Vighi, B. Sala, and others. 2020. Assessment of organophosphate flame retardants in Mediterranean Boops boops and their relationship to anthropization levels and microplastic ingestion. Chemosphere 252: 1–8.

Garcia-Rojas, M. I., K. C. S. Jenner, P. C. Gill, M. N. M. Jenner, A. L. Sutton, and R. D. McCauley. 2018. Environmental evidence for a pygmy blue whale aggregation area in the Subtropical Convergence Zone south of Australia. Mar. Mammal Sci. 34: 901–923.

Gauffier, P., A. Borrell, M. A. Silva, and others. 2020. Wait your turn, North Atlantic fin whales share a common feeding ground sequentially. Mar. Environ. Res. 155: 104884.

Gauffier, P., P. Verborgh, J. Giménez, R. Esteban, J. M. S. Sierra, and R. De Stephanis. 2018. Contemporary migration of fin whales through the Strait of Gibraltar. Mar. Ecol. Prog. Ser. 588: 215–228.

Geijer, C. K. A., G. Notarbartolo di Sciara, and S. Panigada. 2016. Mysticete migration revisited: are Mediterranean fin whales an anomaly? Mamm. Rev. 46: 284–296.

Giménez, J., E. Gómez-Campos, A. Borrell, L. Cardona, and A. Aguilar. 2013. Isotopic evidence of limited exchange between Mediterranean and eastern North Atlantic fin whales. Rapid Commun. Mass Spectrom. 27: 1801–1806.

Godø, O. R., A. Samuelsen, G. J. Macaulay, R. Patel, S. S. Hjøllo, J. Horne, S. Kaartvedt, and J. A. Johannessen. 2012. Mesoscale eddies are oases for higher trophic marine life. PLoS One 7: 1–9.

Heide-Jørgensen, M. P., L. Witting, and M. V Jensen. 2003. Inshore-offshore movements of two fin whales (*Balaenoptera physalus*) tracked by satellite off West Greenland. J. Cetacean Res. Manag. 5: 241–245.

Hilderbrand, G. V., C. T. Robbins, and S. D. Farley. 1998. Response: Use of stable isotopes to determine diets of living and extinct bears. Can. J. Zool. 76: 2301–2303.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: A review. Oecologia 120: 314–326.

Hobson, K. A. 2019. Application of isotopic methods to tracking animal movements, p. 85:115. In K.A. Hobson and L.I. Wassenaar [eds.], Tracking animal migration with stable isotopes. Elsevier Inc.

Hobson, K. A., R. Barnett-Johnson, and T. Cerling. 2010. Using Isoscapes to Track Animal Migration, p. 273–298. In J.B. West, G.J. Bowen, T.E. Dawson, and K.P. Tu [eds.], Isoscapes: Understanding movement, pattern, and process on Earth through isotope mapping. Springer Netherlands.

Hobson, K. A., J. F. Piattt, and J. Pitocchelli. 1994. Using stable isotopes to determine seabird trophic relationships. J. Anim. Ecol. 63: 786–798.

Hobson, K. A., F. F. Riget, P. M. Outridge, R. Dietz, and E. Born. 2004. Baleen as a biomonitor of mercury content and dietary history of North Atlantic Minke Whales (*Balaenoptera acutorostrata*): Combining elemental and stable isotope approaches. Sci. Total Environ. 331: 69–82.

Horton, T. W., N. Hauser, A. N. Zerbini, and others. 2017. Route Fidelity during Marine Megafauna Migration. Front. Mar. Sci. 4: 1–21.

Horwood, J. 2017. Sei Whale, p. 845:847. In B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.], Encyclopedia of Marine Mammals. Academic Press.

International Whaling Comission. 2009. Report of the first intersessional RMP workshop on North Atlantic fin whales. J. Cetacean Res. Manag. 11: 425–452.

Iverson, S. J., and H. N. Koopman. 2018. Blubber, 3rd ed. B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.]. Academic Press.

Jiménez López, M. E., D. M. Palacios, A. Jaramillo Legorreta, and B. R. Mate. 2019. Fin whale movements in the Gulf of California, Mexico, from satellite telemetry. PLoS One 14.

Jonsgård, A. 1966. Biology of the North Atlantic fin whale Balaenoptera physalus (L). Taxonomy. distribution, migration and food. Hvalrådets Skr. 49: 1–62.

Käkelä, R., A. Käkelä, S. Kahle, P. H. Becker, A. Kelly, and R. W. Furness. 2005. Fatty acid signatures in plasma of captive herring gulls as indicators of demersal or pelagic fish diet. Mar. Ecol. Prog. Ser. 293: 191–200.

Kawamura, A. 1980. A review of food of balaenopterid whales. Sci. Reports, Whales Res. Institute, Tokyo 32: 155–197.

Kenney, R. 2017. Right Whales, p. 817–821. In B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.], Encyclopedia of Marine Mammals. Elsevier.

Laws, R. M., and P. E. Purves. 1956. The ear plug of the Mysticeti as an indication of age with special reference to the North Atlantic fin whale (*Balaenoptera physalus* Linn.). Nor. Hvalfangst-Tidende 45: 413–425.

Lesage, V., K. Gavrilchuk, R. D. Andrews, and R. Sears. 2017. Foraging areas, migratory movements and winter destinations of blue whales from the western North Atlantic. Endanger. Species Res. 34: 27–43.

Lockyer, C. H. 1981a. Growth and energy budgets of large baleen whales from the southern hemisphere. Mammals in the seas. v. 3: General papers and large cetaceans. FAO Fish. Ser. 5: 379–487.

Lockyer, C. H. 1981b. Growth and energy budgets of large baleen whales from the southern hemisphere. Mamm. seas 3, General: 379–484.

Lockyer, C. H. 1984. Age determination by means of the earplug in baleen whales. Rep. Int. Whal. Comm. 34: 692–696.

Lübcker, N., R. Condit, R. Beltran, N. de Bruyn, and M. Bester. 2016. Vibrissal growth parameters of southern elephant seals *Mirounga leonina*: obtaining fine–scale, time–based stable isotope data. Mar. Ecol. Prog. Ser. 559: 243–255.

Lydersen, C., J. V. Garcia, M. P. Heide-Jørgensen, N. Øien, C. Guinet, and K. M. Kovacs. 2020. Autumn movements of fin whales (*Balaenoptera physalus*) from Svalbard, Norway, revealed by satellite tracking. Sci. Rep. 10.

Macko, S. A., M. L. Fogel, P. E. Hare, and T. C. Howering. 1987. Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. Chem. Geol. 65: 79–92.

Mahaffey, C., C. R. Benitez-Nelson, R. R. Bidigare, Y. Rii, and D. M. Karl. 2008. Nitrogen dynamics within a wind-driven eddy. Deep. Res. Part II Top. Stud. Oceanogr. 55: 1398–1411.

Maldonado, K., S. D. Newsome, P. Razeto-Barry, J. M. Ríos, G. Piriz, and P. Sabat. 2019. Individual diet specialisation in sparrows is driven by phenotypic plasticity in traits related to trade-offs in animal performance. Ecol. Lett. 22: 128–137.

Martin, A. P., and K. J. Richards. 2001. Mechanisms for vertical nutrient transport within a North Atlantic mesoscale eddy. Deep. Res. Part II Top. Stud. Oceanogr. 48: 757–773.

Mate, B. R., B. A. Lagerquist, and J. Calambokidis. 1999. Movements of North Pacific blue whales during the feeding season off southern California and their southern fall migration. Mar. Mammal Sci. 15: 1246–1257.

Matthews, C. J. D., and S. H. Ferguson. 2013. Spatial segregation and similar trophic-level diet among eastern Canadian Arctic/north-west Atlantic killer whales inferred from bulk and compound specific isotopic analysis. J. Mar. Biol. Assoc. United Kingdom 94: 1343–1355.

McCaffrey, M. A., J. W. Farrington, and D. J. Repeta. 1990. The organic geochemistry of Peru margin surface sediments: I. A comparison of the C37 alkenone and historical El Niño records. Geochim. Cosmochim. Acta 54: 1671–1682.

McClelland, J. W., and J. P. Montoya. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. Ecology 83: 2173–2180.

McGillicuddy, D. J., L. A. Anderson, S. C. Doney, and M. E. Maltrud. 2003. Eddy-driven sources and sinks of nutrients in the upper ocean: Results from a 0.1° resolution model of the North Atlantic. Global Biogeochem. Cycles 17: n/a-n/a.

McHuron, E. A., R. R. Holser, and D. P. Costa. 2018. What's in a whisker? Disentangling ecological and physiological isotopic signals. Rapid Commun. Mass Spectrom. 57–66.

McMahon, K. W., L. L. Hamady, and S. R. Thorrold. 2013a. Ocean ecogeochemistry: a review. Oceanogr. Mar. Biol. An Annu. Rev. 51: 327–374.

McMahon, K. W., L. L. Hamady, and S. R. Thorrold. 2013b. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol. Oceanogr. 58: 697–714.

Mitani, Y., T. Bando, N. Takai, and W. Sakamoto. 2006. Patterns of stable carbon and nitrogen isotopes in the baleen of common minke whale *Balaenoptera acutorostrata* from the western North Pacific. Fish. Sci. 72: 69–76.

Mizroch, S. A., D. W. Rice, and J. M. Breiwick. 1984. The Fin Whale, *Balaenoptera physalus*. Mar. Fish. Rev. 46: 20–24.

Mizroch, S. A., D. W. Rice, D. Zwiefelhofer, J. Waite, and W. L. Perryman. 2009. Distribution and movements of fin whales in the North Pacific Ocean. Mamm. Rev. 39: 193–227.

Morel, F. M. M., A. M. L. Kaepiel, and M. Amyot. 1998. The chemical cycle and bioaccumulation of mercury. Annu. Rev. Ecol. Syst. 29: 543–566.

Nasu, K. 1966. Fishery oceanographic study on the baleen whaling grounds. Sci. Reports Whales Res. Inst. Tokio 20: 157–210.

Newsome, S. D., P. L. Koch, M. A. Etnier, and D. Aurioles-Gamboa. 2006. Using carbon and nitrogen isotope values to investigate maternal strategies in Northeast Pacific otariids. Mar. Mammal Sci. 22: 556–572.

Nieukirk, S. L., D. K. Mellinger, S. E. Moore, K. Klinck, R. P. Dziak, and J. Goslin. 2012. Sounds from airguns and fin whales recorded in the mid-Atlantic Ocean, 1999-2009. J. Acoust. Soc. Am. 131: 1102–1112.

Notarbartolo di Sciara, G., M. Zanardelli, M. Jahoda, S. Panigada, and S. Airoldi. 2003. The fin whale, *Balaenoptera physalus* (L. 1758) in the Mediterranean Sea. Mamm. Rev. 33: 105–150.

Panigada, S., G. P. Donovan, J.-N. Druon, and others. 2017. Satellite tagging of Mediterranean fin whales: working towards the identification of critical habitats and the focussing of mitigation measures. Sci. Rep. 7: 3365.

Pereira, A., D. Harris, P. L. Tyack, and L. Matias. 2020. Fin whale acoustic presence and song characteristics in seas to the southwest of Portugal. J. Acoust. Soc. Am. 147: 2235–2249.

Pérez-Jorge, S., M. Tobeña, R. Prieto, F. Vandeperre, B. Calmettes, P. Lehodey, and M. A. Silva. 2020. Environmental drivers of large-scale movements of baleen whales in the mid-North Atlantic Ocean. Divers. Distrib. 26: 683–698.

Perrin, W. F., S. D. Mallefte, and R. L. Brownell. 2017. Minke whales, p. 608:613. In B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.], Encyclopedia of Marine Mammals. Academic Press.

Podlesak, D. W., S. R. McWilliams, and K. A. Hatch. 2005. Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. Oecologia 142: 501–510.

Popp, B. N., B. S. Graham, R. J. Olson, C. C. S. Hannides, M. J. Lott, G. A. López-Ibarra, F. Galván-Magaña, and B. Fry. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. Terr. Ecol. 1: 173–190.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montaña. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152: 179–189.

Prahl, F. G., L. A. Muehlhausen, and D. L. Zahnle. 1988. Further evaluation of long-chain alkenones as indicators of paleoceanographic conditions. Geochim. Cosmochim. Acta 52: 2303–2310.

Prahl, F. G., and S. G. Wakeham. 1987. Calibration of unsaturation patterns in long-chain ketone compositions for palaeotemperature assessment. Nature 330: 367–369.

Ramos, R., and J. González-Solís. 2012. Trace me if you can: The use of intrinsic biogeochemical markers in marine top predators. Front. Ecol. Environ. 10: 258–266.

Rice, D. W. 2002. Baleen, p. 61–62. In W.F. Perrin, B. Würsig, and J.G.M. Thewissen [eds.], Encyclopedia of Marine Mammals. Elsevier-Academic Press.

Richard, G., O. V. Titova, I. D. Fedutin, and others. 2018. Cultural transmission of fine-scale fidelity to feeding sites may shape humpback whale genetic diversity in Russian pacific waters. J. Hered. 109: 724–734.

Rita, D., A. Borrell, and A. Aguilar. 2021. Alkenones in oceanic odontocetes as a potential proxy of environmental water temperature. Ecol. Indic. 122.

Robinson, E. M., S. J. Trumble, B. Subedi, R. Sanders, and S. Usenko. 2013. Selective pressurized liquid extraction of pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in a whale earplug (earwax): A novel method for analyzing organic contaminants in lipid-rich matrices. J. Chromatogr. A 1319: 14–20.

Roe, H. S. J. 1967. Seasonal formation of laminae in the ear of the fin whale. Discov. reports 35: 1–30.

Romagosa, M., M. Baumgartner, I. Cascão, M. O. Lammers, T. A. Marques, R. S. Santos, and M. A. Silva. 2020. Baleen whale acoustic presence and behaviour at a Mid-

Atlantic migratory habitat, the Azores Archipelago. Sci. Rep. 10: 1–11. doi:10.1038/s41598-020-61849-8

Ruiz-Cooley, R. I., P. L. Koch, P. C. Fiedler, and M. D. McCarthy. 2014. Carbon and nitrogen isotopes from top predator amino acids reveal rapidly shifting ocean biochemistry in the outer California current. PLoS One 9.

Ryan, C., S. D. Berrow, B. Mchugh, C. O'Donnell, C. N. Trueman, and I. O'Connor. 2014. Prey preferences of sympatric fin (*Balaenoptera physalus*) and humpback (*Megaptera novaeangliae*) whales revealed by stable isotope mixing models. Mar. Mammal Sci. 30: 242–258.

Sanpera, C., and A. Aguilar. 1992. Modern whaling off the Iberian Peninsula during the 20th century. Rep. - Int. Whal. Comm. 42: 723–730.

Sears, R., and W. F. Perrin. 2017. Blue Whale, p. 110:114. In B. Würsig, J.G.M. Thewissen, and K.M. Kovacs [eds.], Encyclopedia of Marine Mammals. Academic Press.

Siegel, V. 2000. Krill (Euphausiacea) demography and variability in. Can. J. Fish. Aquat. Sci. 57: 151–167.

Sigurjónsson, J., and G. A. Víkingsson. 1997. Seasonal abundance of and estimated food consumption by cetaceans in Icelandic and adjacent waters. J. Northwest Atl. Fish. Sci. 22: 271–287.

Silva, M. A., A. Borrell, R. Prieto, P. Gauffier, M. Bérubé, P. J. Palsbøl, and A. Colaço. 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. R. Soc. Open Sci. 6.

Silva, M. A., R. Prieto, I. Jonsen, M. F. Baumgartner, and R. S. Santos. 2013. North Atlantic blue and fin whales suspend their spring migration to forage in middle latitudes: building up energy reserves for the journey? PLoS One 8.

Skov, H., T. Gunnlaugsson, W. P. Budgell, and others. 2008. Small-scale spatial variability of sperm and sei whales in relation to oceanographic and topographic features along the Mid-Atlantic Ridge. Deep. Res. Part II Top. Stud. Oceanogr. 55: 254–268.

Soule, D. C., and W. S. D. Wilcock. 2013. Fin whale tracks recorded by a seismic network on the Juan de Fuca Ridge, Northeast Pacific Ocean. J. Acoust. Soc. Am. 133: 1751–1761.

Stafford, K. M., D. K. Mellinger, S. E. Moore, and C. G. Fox. 2007. Seasonal variability and detection range modeling of baleen whale calls in the Gulf of Alaska, 1999–2002. J. Acoust. Soc. Am. 122: 3378–3390.

Svanbäck, R., and D. I. Bolnick. 2007. Intraspecific competition drives increased resource use diversity within a natural population. Proc. R. Soc. B Biol. Sci. 274: 839–844.

Troisi, G. M., K. Haraguchi, D. S. Kaydoo, M. Nyman, A. Aguilar, A. Borrell, U. Siebert, and C. F. Mason. 2000. Bioaccumulation of polychlorinated biphenyls (PCBs) and dichlorodiphenylethane (DDE) methyl sulfones in tissues of seal and dolphin morbillivirus epizootic victims. J. Toxicol. Environ. Heal. Part a 62: 1–8.

Vighi, M., A. Borrell, and A. Aguilar. 2017. Bone as a surrogate tissue to monitor metals in baleen whales. Chemosphere 171: 81–88.

Vighi, M., A. Borrell, G. Víkingsson, T. Gunnlaugsson, and A. Aguilar. 2019. Strontium in fin whale baleen: A potential tracer of mysticete movements across the oceans? Sci. Total Environ. 650: 1224–1230.

Víkingsson, G. A., and M. P. Heide-Jørgensen. 2015. First indications of autumn migration routes and destination of common minke whales tracked by satellite in the North Atlantic during 2001–2011. Mar. Mammal Sci. 31: 376–385.

Vikingsson, G. A., D. G. Pike, G. Desportes, N. Oien, T. Gunnlaugsson, and D. Bloch. 2009. Distribution and abundance of fin whales (*Balaenoptera physalus*) in the Northeast and Central Atlantic as inferred from the North Atlantic Sightings Surveys 1987-2001. NAMMCO Sci. Publ. 7: 49–72.

Víkingsson, G. A., D. G. Pike, H. Valdimarsson, and others. 2015. Distribution, abundance, and feeding ecology of baleen whales in Icelandic waters: Have recent environmental changes had an effect? Front. Ecol. Evol. 3.

Warinner, C., and N. Tuross. 2010. Tissue isotopic enrichment associated with growth depression in a pig: Implications for archaeology and ecology. Am. J. Phys. Anthropol. 141: 486–493.

Watkins, W. a, J. Sigurjónsson, D. Wartzok, R. R. Maiefski, P. W. Howey, and M. a Daher. 1996. Fin whale tracked by satellite off Iceland. Mar. Mammal Sci. 12: 564–569.

Whooley, P., S. Berrow, and C. Barnes. 2011. Photo-identification of fin whales (*Balaenoptera physalus* L.) off the south coast of Ireland. Mar. Biodivers. Rec. 4: 1–7.

Woo, K. J., K. H. Elliott, M. Davidson, A. J. Gaston, and G. K. Davoren. 2008. Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. J. Anim. Ecol. 77: 1082–1091.

Vander Zanden, H. B., K. A. Bjorndal, and A. B. Bolten. 2013. Temporal consistency and individual specialization in resource use by green turtles in successive life stages. Oecologia 173: 767–777.

Vander Zanden, H. B., A. B. Bolten, A. D. Tucker, and others. 2016. Biomarkers reveal sea turtles remained in oiled areas following the Deepwater Horizon oil spill. Ecol. Appl. 26: 2145–2155.