UNIVERSITAT DE BARCELONA

Aplicación de técnicas moleculares y químicas al estudio de la dieta, migración y estructura poblacional del rorcual común de Islandia

Raquel García Vernet

RAQUEL GARCÍA VERNET

APLICACIÓN DE TÉCNICAS MOLECULARES Y QUÍMICAS AL ESTUDIO DE LA DIETA, MIGRACIÓN Y ESTRUCTURA POBLACIONAL DEL RORCUAL COMÚN DE ISLANDIA

Cita recomendada:

García-Vernet, R. (2022) Aplicación de técnicas moleculares y químicas al estudio de la dieta, migración y estructura poblacional del rorcual común de Islandia. Tesis doctoral. Universidad de Barcelona, Facultad de Biología.

La ilustración de la portada y las ilustraciones iniciales de capítulo son creación de Camila Suaya (@mad.cami).

Facultad de Biología
Programa de doctorado en Biodiversidad

Departamento de Biología Evolutiva, Ecología y Ciencias Ambientales
Departamento de Genética, Microbiología y Estadística

Aplicación de técnicas moleculares y químicas al estudio de la dieta, migración y estructura poblacional del rorcual común de Islandia

Memoria presentada por

Raquel García Vernet

para optar al grado de doctora por la Universidad de Barcelona

Doctoranda
Raquel García Vernet

Directora y Tutora
Assumpció Borrell Thió

Directora
Marta Riutort León

ABSTRACT

The fin whale is one of the most common mysticetes inhabiting the North Atlantic and, as most baleen whales, undertakes annual migrations alternating high-latitude summer feeding grounds with low-latitude winter breeding grounds. Most of the information about the fin whale is collected during summer periods, when this species aggregates to feed. However, once the fin whale starts its migration and departs from the feeding grounds, there is an important gap of knowledge involving its migratory strategy, diet, and population structure. The main objective of this thesis is to expand the knowledge about the fin whale biology and ecology, focusing on the Icelandic fin whale population. To this aim, $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values were determined in skin and baleen plate samples, to obtain information about fin whale trophic ecology and migratory patterns. Also, DNA methylation was analyzed in skin samples of Icelandic and Spanish fin whales, to better understand the differentiation between both populations, and to also test the use of methylation levels to determine age of individuals. Results highlight that Icelandic fin whales strongly rely on krill during summer. In addition, a significant overlap of isotopic niches was detected between fin and blue whales. This interspecific competition may be mitigated by some degree of spatio-temporal segregation in the Icelandic waters, reflected in the $\delta^{34}S$ values of both species. The information provided by the stable isotopes analyzed in baleen plates showed that most fin whales perform annual migrations, which were also reflected in their $\delta^{34}S$ values. $\delta^{15}N$ values showed high inter-individual variability, suggesting that fin whales disperse during winter. However, we found some pairs of individuals with no kinship showing nearly identical isotopic patterns for two consecutive years, indicating of long-term association. Finally, methylation analyses showed that three genes presented age-related variation, but these results did not allow to perform accurate epigenetic age estimations. In addition, fin whale populations from Iceland and Spain exhibited differentially methylated regions throughout their epigenomes. A considerable number of the genes associated to these regions were related to the circadian clock or other characteristics related to migration, probably reflecting differential migrations between individuals of both populations. Overall, results of this thesis contribute to better understand the behavior, ecology and population structure of the North Atlantic fin whale.

AGRADECIMIENTOS

Y al fin, parece que ha llegado. Si soy sincera, todavía no me creo que esté escribiendo la sección de agradecimientos. Los que me conocen saben que ha sido un camino largo (y, en ocasiones, arduo), pero parece que ha llegado el momento de poner punto (¿y aparte?) a esta etapa.

Querría empezar agradeciendo a mis dos codirectoras de tesis. Agradecerle a Xon, no solamente por ser una gran profesional y científica, sino también por su apoyo en los momentos en los que más los he necesitado. Agradecerle también a Marta Riutort, que me acogió por primera vez durante el TFG y consiguió que me ilusionara por la ciencia gracias a su dedicación y motivación. Muchas gracias a ambas por la profesionalidad, la paciencia, la disponibilidad y los ánimos, que llegaron incluso en los momentos más complicados. Tengo muy claro que esta tesis no estaría aquí si no hubiera sido por vosotras.

Aunque sobre el papel he tenido dos directoras, no quiero olvidarme de mi director en las sombras. Alex, muchas gracias por haberme dado la oportunidad de estar en el grupo y por el tiempo que has dedicado a todos mis trabajos. Aunque sé que en ocasiones ha sido un reto entendernos, tengo claro que los trabajos no serían lo que son si no hubiera sido por ti, y sé que he aprendido muchas cosas (entre ellas ¡espero que escribir artículos!) gracias a ti.

Agradecer también a todos los servicios técnicos que me han permitido realizar la mayoría de los análisis de esta tesis doctoral. Gracias al CCiT-UB (Centros Científicos y Tecnológicos de la UB), en concreto a las unidades de Genómica y de Espectrometría. Me gustaría agradecer especialmente a Amaya, por su increíble soporte durante la preparación y secuenciación de las librerías RRBS. También me gustaría agradecer a Joaquim, Rosa Maria y Pili, por su insustituible ayuda durante la realización de los análisis isotópicos.

Además de mis directores y el soporte técnico, mucha más gente ha sido partícipe de este proceso. Me gustaría empezar agradeciendo a todos los miembros del grupo de Grandes Vertebrados Marinos, con los que he compartido cafés, reuniones y congresos a lo largo de este tiempo: Manel, Lluis, Morgana, Patricia, Maria, Massimo, Alessandra, Jazel y Marina Arregui. Me gustaría lanzar una especial dedicatoria a los que han sido mis compañeros principales durante prácticamente todos estos años: Odei, Diego, hemos compartido dramas, más dramas, congresos (¡y fiestas de congresos!), cafés, room escapes… ¡Y espero que lo sigamos haciendo! También agradecer a los que han sido mis estudiantes de TFG y TFM (Pol, Julia Elgueta, Julia Zafra, Marina Pascual, Elena), que han contribuido enormemente en algunos de los trabajos que aquí se presentan.

Llega el turno de los miembros del grupo de FilMol y la sección de Genética. Muchas gracias a Edu, Ona, Joan, Gemma (¡las PCRs no habrían salido si no fuera por ti!). A Cristian, por su increíble ayuda con la bioinformática y las risas con los botones exterminadores. A Dani, por compartir cafés y dejarme dibujos de ballenitas que me alegraban la tarde. A Laia y a Lisi, por todas las charlas, las risas y las terapias de

dramas (¡dentro y fuera del lab!). A Ricard (siempre serás del lab FilMol), por haber sido un gran amigo durante todos estos años y alegrarme siempre que nos vemos. A Carlos, por compartir conversaciones (muy) frikis... ¡tenemos pendiente jugar a las mansiones!

Y… ¡no me olvido de vosotros! Paula, gracias por haber sido uno de mis máximos apoyos durante toda la tesis. Es curioso, pero desde que nos conocimos haciendo el TFG (¡aún recuerdo el inicio de aquella conversación super friki!) hemos compartido prácticamente el mismo camino. Has sido una de las personas que ha estado desde el principio hasta el final, y solamente puedo darte las gracias por todo. Gaspar, gracias por haberme alegrado tantos y tantos días, y por haberme entendido tan bien incluso cuando nos conocíamos tan poco. Gracias por los cafés, los ratos tirados en el parque, las risas y, en definitiva, todos los ratillos que hemos compartido juntos.

Me gustaría también agradecer a toda la gente que he conocido en Doctorandes en Lluita, por haberme enseñado tantas cosas, entre ellas a no rendirme pase lo que pase. Aunque he conocido a mucha gente maravillosa, me gustaría dar una especial dedicatoria a Berta: muchas gracias por todos los cafés, la experiencia Martinet, y, sobre todo, por contagiar tanta felicidad.

Muchas gracias también a la gente de fuera del ambiente de la investigación que, aunque en muchas ocasiones no entendían de qué iba esto de la tesis, siempre me han apoyado de manera incondicional. Gracias a mis amigos “originals”, en especial a Alex, Enric, Pol y Carlos R., por los momentos de risas, birras, desconexión y ánimos. A los Ondras, por sacarme a la montaña en momentos de máximo estrés y caos. A Nuria y Marina Roldán, por escucharme hablar de la tesis durante tantísimas horas. Y finalmente, ¡Camila! Estoy bastante segura de que eres de las personas que más ha escuchado hablar de mi tesis. Muchísimas gracias por todo el apoyo incondicional que me has dado durante todos estos años, por las aventuras y las risas. Estoy muy contenta de que esta tesis haya podido llevar parte de tu arte.

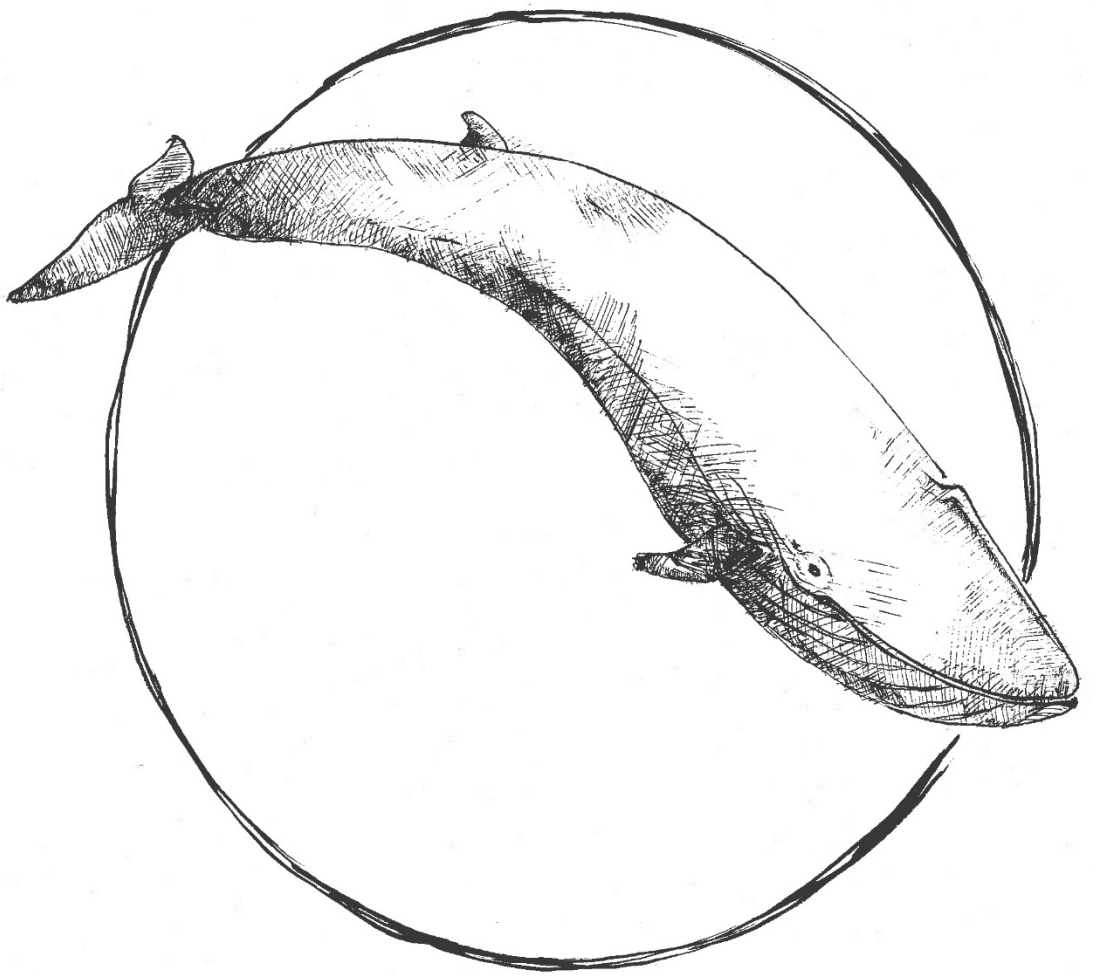
No puedo acabar los agradecimientos sin agradecer a mi familia y a mi pareja. Muchas gracias a mis tíos, primitos y a mi yaya, por demostrar que la familia siempre está ahí. En especial agradecer a mis padres, no solamente por el apoyo durante esta tesis, sino durante todos estos años. Gracias también a Pol y Sonia, por su felicidad contagiosa y por ser los mejores hermanos del mundo.

Y, finalmente, a Martí. Sé que muchas veces no has entendido qué narices estaba haciendo con mi vida. Aun así, has estado ahí para mí y me has apoyado incondicionalmente, y estoy segurísima de que esta tesis no sería lo que es si no fuera por ti. Muchas gracias por todos los momentos que hemos pasado juntos y, sobre todo, por haber estado conmigo en el que ha sido el momento más complicado de esta tesis. Sé que si lees esto te morirás de vergüenza, pero como es mi tesis hago lo que quiero, y lo que quiero decirte es que ahora que ya he depositado… ¡por fin podremos ir a escalar juntos de nuevo!

En fin, muchas gracias a todos y todas los que habéis estado conmigo en algún momento de este camino. Esta tesis está aquí gracias a vosotros.

ÍNDICE

ÍNDICE



INTRODUCCIÓN

INTRODUCCIÓN

Marco general de la tesis

El rorcual común (*Balaenoptera physalus*) es una especie cosmopolita, y una de las ballenas más abundantes del Atlántico Norte. Al igual que otros misticetos, efectúa migraciones estacionales que alternan latitudes altas y productivas en verano, donde se concentran un gran número de individuos, con latitudes bajas y menos productivas en invierno. Aprovechando las agrupaciones que suceden en verano, el rorcual común fue vastamente explotado por la industria ballenera en las zonas de alimentación, hasta que entró en vigor la moratoria en el año 1985.

A pesar de su explotación histórica y su relativa abundancia en el Atlántico norte, siguen existiendo multitud de incógnitas, especialmente relacionadas con su ecología y comportamiento fuera de las zonas de alimentación. En esta tesis se pretenden resolver algunas de las preguntas que existen entorno a esta especie, que serán planteadas a lo largo de la sección de Introducción y posteriormente redefinidas en la sección de Objetivos. Para ello se focalizará el análisis en la población de rorcual común de Islandia, y se utilizarán principalmente análisis químicos (análisis de isótopos estables) y análisis genéticos (principalmente análisis de los patrones de metilación del ADN) para tratar de resolver algunas de estas incógnitas.

1. El rorcual común

1.1. Los rorcuales. Contexto taxonómico general

Los misticetos, comúnmente conocidos como ballenas barbadas, son uno de los dos subórdenes de los cetáceos existentes en la actualidad. Todas las especies comprendidas dentro de este suborden se caracterizan principalmente por la ausencia de dientes funcionales. Éstos han sido reemplazados por unas estructuras queratinosas, comúnmente conocidas como barbas, que cuelgan de la encía de la mandíbula superior y permiten la filtración de pequeños organismos presentes en la columna de agua (Bannister, 2018).

Las relaciones filogenéticas existentes entre las especies comprendidas dentro del suborden de los misticetos, así como su clasificación taxonómica, han sido sujeto de estudio a lo largo de los últimos años, presentando ciertas discordancias según la metodología utilizada (Árnason et al. 2004, Mc Gowen et al. 2009, Steeman et al. 2009,

Marx and Fordyce 2015, Árnason et al. 2018). Actualmente, dentro del suborden de los misticetos se reconocen 4 familias distintas, establecidas a partir del análisis de características anatómicas (Bannister, 2018):

i) **Balaenidae**, que incluye dos géneros: *Balaena*, con una única especie (la ballena de Groenlandia, *Balaena mysticetus*), y *Eubalaena*, con tres especies distintas comúnmente conocidas como ballenas francas.

ii) **Neobalenidae**, que incluye a la ballena franca pigmea (*Caperea marginata*).

iii) **Eschrichtiidae**, que incluye a la ballena gris (*Eschrichtius robustus*).

iv) **Balaenopteridae**, que incluye dos géneros: *Megaptera*, con una única especie representante (la ballena jorobada, *Megaptera novaeangliae*), y *Balaenoptera*.

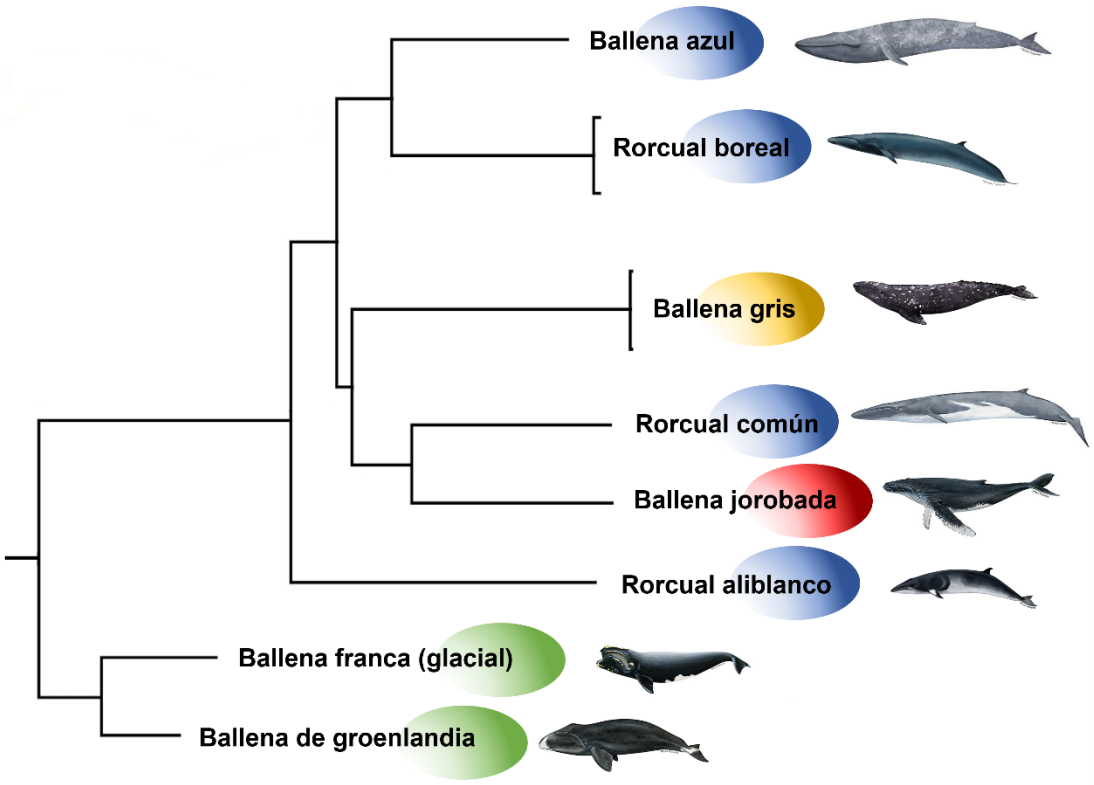


***Figura 1**: Filogenia adaptada de Árnason et al. 2018, construida a partir de datos genómicos. Aunque se trata de una de las filogenias más robustas, no se incluyen datos de la familia Neobalenidae ni de algunas especies de rorcuales. Los colores muestran las distintas familias y géneros: en verde, la familia Balaenidae; en amarillo, la familia Eschrichtiidae; en rojo, el género Megaptera; y finalmente en azul, el género Balaenoptera. Las figuras de cada una de las especies han sido obtenidas de la página web de la NOAA.*

En general, las últimas filogenias apuntan a que la divergencia de la familia Balaenopteridae, que incluye los géneros *Balaenoptera* y *Megaptera*, se produjo hace 10 – 15 millones de años (Mc Gowen et al. 2009, Árnason et al. 2018, McGowen et al. 2020). Las especies comprendidas dentro del género *Balaenoptera* son las que se conocen comúnmente como rorcuales, dada su morfología estilizada y los pliegues presentes en la garganta (Bannister, 2018). Aunque actualmente este género comprende 8 especies, este número podría cambiar en los próximos años debido a la inclusión de nuevas especies (Wada et al. 2003, Rosel et al. 2021), así como a la reasignación taxonómica de especies ya existentes (Árnason et al. 2018, McGowen et al. 2020).

De manera contraintuitiva, y a pesar de las claras diferencias morfológicas, la mayoría de los análisis moleculares, realizados tanto con marcadores nucleares como con marcadores mitocondriales, resuelven de manera robusta que la especie más próxima al rorcual común (*Balaenoptera physalus*) es la ballena jorobada (Sasaki et al. 2005, Slater et al. 2010, Mc Gowen et al. 2009, Árnason et al. 2018), convirtiendo el género *Balaenoptera* en parafilético. Así mismo, la familia Balaenopteridae también podría ser parafilética, ya que filogenias recientes sitúan a la ballena gris (familia Eschrichtiidae) dentro de este grupo (Figura 1, Árnason et al. 2018, McGowen et al. 2020).

1.2. El rorcual común, ciclo reproductivo y migraciones

El rorcual común (*Balaenoptera physalus*) es una de las especies reconocidas actualmente dentro del género *Balaenoptera*. Es un cetáceo cosmopolita (Edwards et al. 2015), aunque mayoritariamente se localiza en aguas templadas o frías.

Se trata de una especie longeva, habiéndose descrito individuos de hasta 80 – 90 años (Aguilar and García-Vernet, 2018). Alcanza la madurez sexual alrededor de los 6 – 8 años, y el máximo tamaño corporal a los 9 – 13 años, siendo las hembras algo más tardías y grandes que los machos (Aguilar and Lockyer, 1987). El ciclo reproductivo es bianual, y como se verá posteriormente, está estrechamente vinculado a las migraciones y movimientos estacionales que ocurren en esta especie. El periodo de apareamiento es en invierno, y la gestación suele durar alrededor de 11 meses, generalmente dando a luz a una sola cría (Aguilar and García-Vernet 2018, Mizroch et al. 1984). El periodo de lactancia dura unos 6-7 meses, y durante éste se establece una fuerte relación entre madre y cría, que es considerada el único vínculo social que ocurre en esta especie (Aguilar and García-Vernet 2018). Este vínculo se rompe cuando se produce el destete

y la cría comienza a alimentarse de forma independiente, tras lo cual la hembra tendrá una fase de reposo de 6 meses hasta el siguiente periodo de reproducción.

Las migraciones se construyen alrededor de este ciclo reproductivo. Como en otras especies de ballenas, la mayoría de los individuos de rorcual común parecen efectuar migraciones estacionales, alternando latitudes altas y productivas en verano con latitudes más bajas y menos productivas en invierno (Lockyer and Brown 1981). Tanto la cópula como el parto, que se producen en invierno, ocurrirán en zonas de aguas calmadas y templadas, mientras que el destete ocurrirá en las zonas productivas, donde la cría podrá comenzar a alimentarse de manera autónoma (Figura 2). Además de esto, la cría efectuará su primera migración acompañada por su madre, dándole la oportunidad de aprender no solamente las zonas de alimentación y reproducción, sino también las rutas migratorias (Whitehead and Rendell 2015).

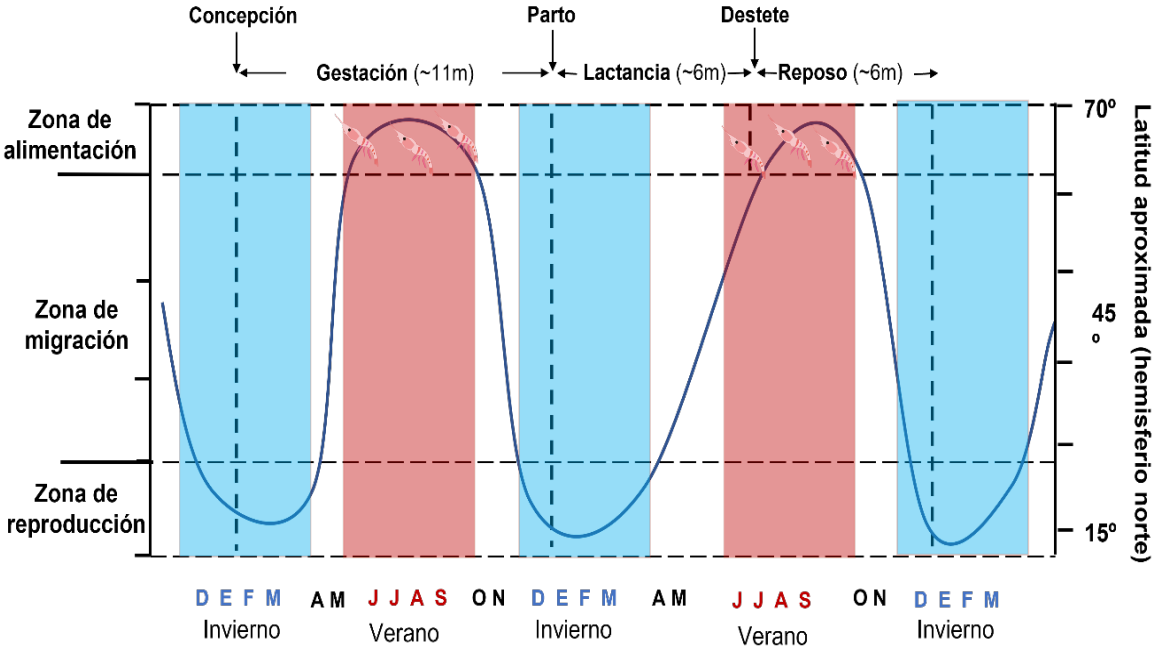


Figura 2: *Ciclo reproductor arquetípico de las ballenas. La concepción se produce durante el invierno y, tras 11 meses, se produce el parto. El periodo de lactancia ocurre principalmente en latitudes bajas. La migración de las hembras lactantes acompañadas por crías es más lenta que la de las hembras gestantes o en reposo. Finalmente, el destete se produce en verano, en las zonas de alimentación. Figura adaptada para esta tesis, a partir de Lockyer and Brown 1981, Bannister 2018.*

Aunque queda patente la importancia que tienen las migraciones dentro del ciclo reproductivo de las ballenas, la falta de información resulta abrumadora. Dentro de la

familia Balaenopteridae, la mayoría de los estudios se han centrado en la ballena jorobada, que parece que cumple el arquetipo de migración previamente descrito.

En la ballena jorobada, los individuos se agrupan tanto en verano, en las zonas de alimentación, como en invierno, en las zonas de reproducción y cría, y basándose en estas últimas se han podido delimitar e identificar poblaciones (Bettridge 2015, Clapham 2018). Además, distintos estudios genéticos han evidenciado que las ballenas jorobadas muestran una fuerte fidelidad a las zonas de alimentación y reproducción de la madre, demostrando así que, en esta especie, la transmisión cultural configura la estructura poblacional (Baker et al. 2013, Barendse et al. 2013, Richard et al. 2018).

Al igual que las ballenas jorobadas, la mayoría de las especies del género *Balaenoptera* también se agrupan durante las épocas de verano, en zonas productivas de latitudes altas. Sin embargo, en las migraciones estacionales muestran una variabilidad interindividual mucho mayor (ver por ejemplo Lydersen et al. 2020), y se ha sugerido que los individuos puedan estar dispersándose durante el invierno (Mackintosh 1966, Payne and Webb 1971, Whitehead and Rendell 2015). Esta escasa agrupación en las zonas de reproducción dificulta mucho el estudio de estas especies, así como la inferencia de su estructura poblacional, provocando que exista poca información más allá de la recopilada en las zonas de alimentación.

1.3. Estructura poblacional del rorcual en el Atlántico Norte

El rorcual común era una de las ballenas más abundantes del Atlántico norte, y a partir de finales del siglo XIX fue una de las especies objetivo de la caza comercial junto con otras especies de grandes cetáceos (Clapham and Baker 2018). Aunque el número de capturas no fue tan dramático como en el hemisferio sur, en el Atlántico Norte se concentraron un gran número de explotaciones, hasta que en el año 1985 entró en vigor la moratoria establecida por la “International Whaling Commission” (IWC).

A diferencia de otras especies cercanas, como por ejemplo la ballena azul, el rorcual común parece estarse recuperando de la explotación ballenera (Víkingsson et al. 2009, Tomas et al. 2016, Cook 2018a). Actualmente se estima que la población en el Atlántico norte es de alrededor de unos 79.000 individuos (Cook 2018b). Esta cifra no incluye las costas irlandesas ni el mar Mediterráneo, cuya población de rorcuales comunes podría rondar los 3500 individuos (Forcada et al. 1996, Reeves and Notarbartolo di Sciara 2006).

A pesar de estas tendencias al alza, cabe destacar que la recuperación del rorcual común no ha sido homogénea en todo el océano. Por ejemplo, a principios del siglo XX se explotó y erradicó una población aparentemente residente del estrecho de Gibraltar (Sanpera and Aguilar 1992). Aún con el cese de la caza y la subsecuente recuperación en el resto del Atlántico, la población del estrecho de Gibraltar parece estar virtualmente extinta, y no se ha producido una reocupación de la zona (Clapham et al. 2008). Estas recuperaciones asimétricas, tanto entre especies como entre poblaciones, dibujan un escenario complejo afectado tanto por la ecología como por la estructura poblacional y la transmisión cultural de las especies afectadas.

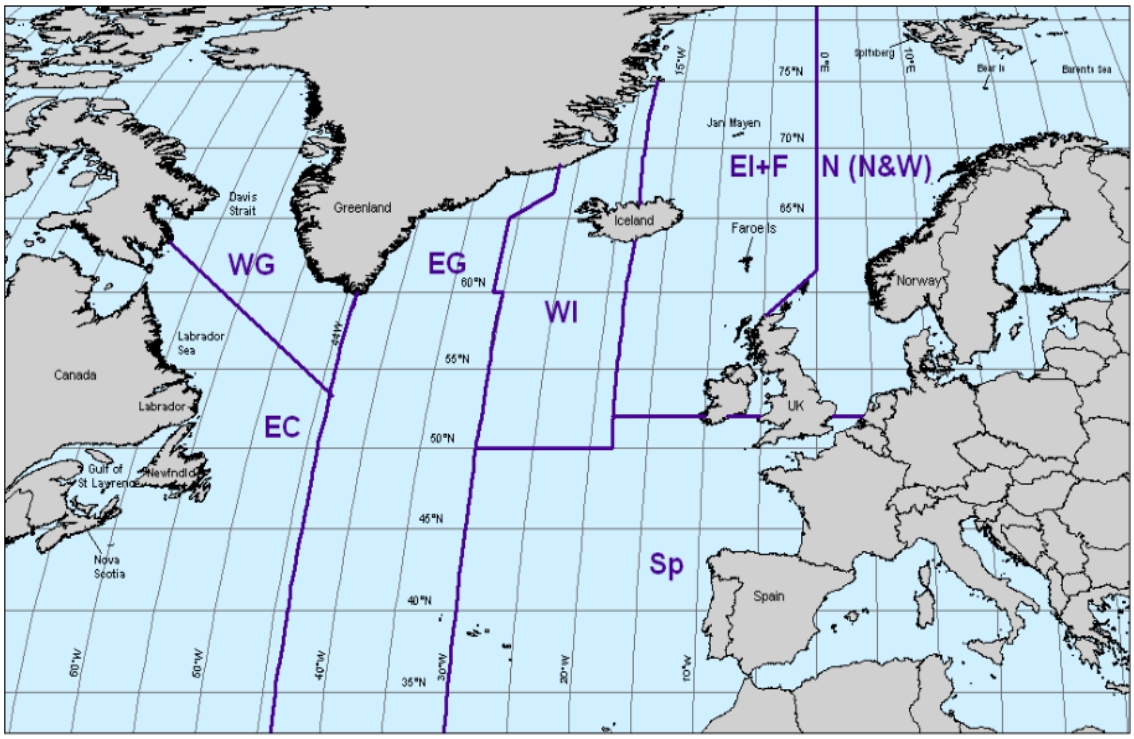


***Figura 3**: Los 7 stocks reconocidos en el Atlántico norte, que delimitan las distintas zonas de alimentación. EC= Este de Canadá + Este USA; WG= Oeste de Groenlandia; EG= Este de Groenlandia; WI = Oeste de Islandia; EI + F = Este de Islandia + Islas Feroe; N= Noruega; Sp= España. Figura extraída de IWC (2009).*

La estructura poblacional del rorcual común ha sido sujeto de interés desde los inicios de la IWC. Basándose principalmente en las capturas de las explotaciones balleneras (Donovan 1991), junto con estudios genéticos (revisado en Pampoulie and Danielsdottir 2013), marcajes (revisado en IWC 2009) y otras fuentes de datos (por ejemplo, Jover 1992, Víkingsson et al 2005), la IWC delimitó 7 stocks en el Atlántico Norte (IWC 2009, Figura 3). Estos stocks reflejan principalmente la separación entre las distintas zonas de

alimentación que explota el rorcual común durante el verano. Sin embargo, la mayoría de los estudios disponibles no proporcionan apenas información sobre la estructura poblacional en las zonas de reproducción, ocupadas durante el invierno (IWC 2009). En ese mismo informe, la IWC sugiere diversas hipótesis sobre las zonas de reproducción, siendo la más factible la existencia de 4 stocks reproductivos (Figura 4).

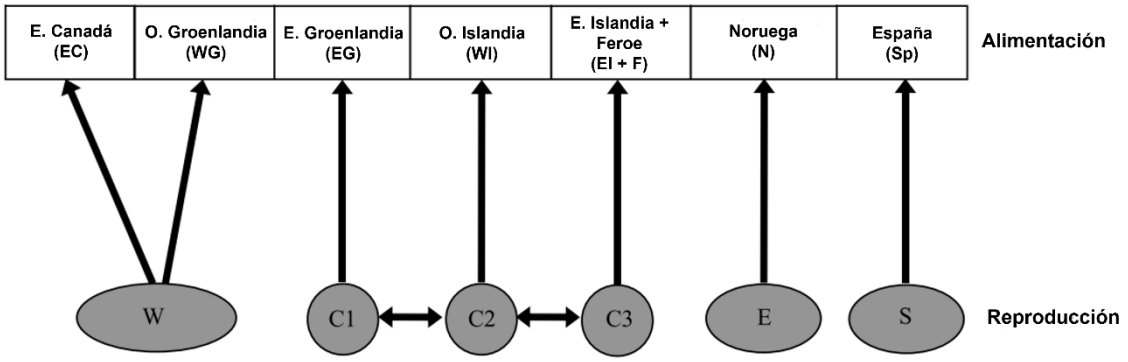


***Figura 4**: Los 7 stocks reconocidos en el Atlántico norte, que delimitan las distintas zonas de alimentación, con una de sus posibles agrupaciones en la época reproductiva. Al contrario que los stocks de alimentación, la composición de la mayoría de los stocks de reproducción está poco respaldada. Las siglas de las zonas de alimentación corresponden a: EC= Este de Canadá + Este USA; WG= Oeste de Groenlandia; EG= Este de Groenlandia; WI = Oeste de Islandia; EI + F = Este de Islandia + Islas Feroe; N= Noruega; Sp= España. Las siglas de las zonas de reproducción corresponden a: W= stock oeste; C= stock central (dividido en tres substocks); E= stock este; S= stock de España. Figura extraída de IWC (2009).*

2. Islandia

2.1. Ballenas en Islandia

Islandia se localiza en el Atlántico Norte, formando parte de la dorsal Mesoatántica. La isla está rodeada de una plataforma continental de más de 100.000 km^2, cuya profundidad varía entre los 200 y 500 metros (Símonarson et al. 2020). Las corrientes marinas que la rodean son complejas: por un lado, recibe aportes de agua relativamente caliente y salina de la corriente de Irminger; por otro lado, recibe agua polar, fría y baja en salinidad de las corrientes del Este de Groenlandia y del Este de Islandia (Símonarson et al. 2020, Figura 5). Las interacciones entre estas corrientes y su variación anual, relacionada con el deshielo y los cambios de temperatura, están estrechamente vinculadas con las altas concentraciones de clorofila que encontramos en aguas islandesas.

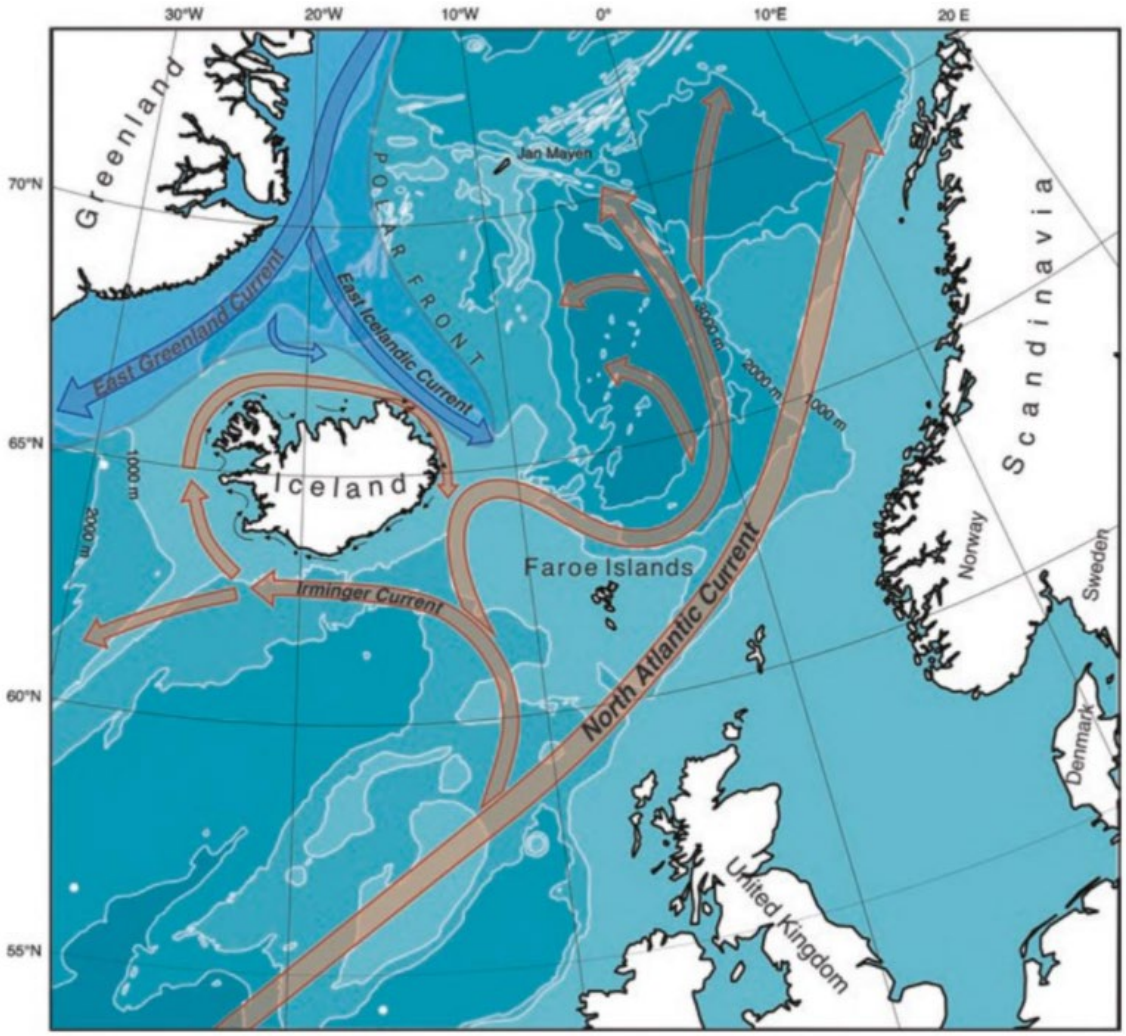


***Figura 5**: Circulación oceánica que afecta a las aguas islandesas. En rojo puedes apreciarse las corrientes más cálidas que proceden del Atlántico norte, mientras que en azul se indican las corrientes más frías procedentes del polo. Mapa extraído de Símonarson et al. (2020).*

El ciclo anual de clorofila en Islandia presenta un pico pronunciado en primavera, que se produce a principios de abril en el norte de la isla y a mediados de mayo en el sur (Zhai et al. 2012). Estos picos de producción primaria convierten las aguas islandesas en zonas ricas, atrayendo a sus aguas distintas especies de ballenas. En Islandia podemos encontrar cinco de las especies que conforman la familia Balaenopteridae: la ballena azul (*Balaenoptera musculus*), el rorcual común, el rorcual boreal (*Balaenoptera borealis*), el rorcual aliblanco (*Balaenoptera acutorostrata*) y la ballena jorobada.

Aunque los tiempos de llegada y permanencia en las aguas islandesas pueden variar ligeramente según la especie, el pico de abundancia de todas ellas oscila entre junio y

agosto (Sigurjónsson and Víkingsson 1997). Añadido a esta coincidencia temporal, las necesidades ecológicas de todas ellas son bastante similares, lo que debería implicar algún grado de competición interespecífica (Mori and Butterworth 2006). Por tanto, resulta intrigante cómo estas cinco especies son capaces de coexistir en las mismas aguas y repartirse los recursos disponibles, evitando así la competencia exclusiva.

Actualmente existen estudios detallados sobre la dieta en Islandia de las especies que han sido explotadas comercialmente en los últimos años: el rorcual común (Víkingsson 1997) y el rorcual aliblanco (Víkingsson et al. 2015). Sin embargo, para el resto de las especies la información es mucho más escasa, y está basada en estudios realizados en otras zonas (ver por ejemplo Gavrilchuk et al. 2014, Wright et al. 2016) o a raíz de observar asociaciones recurrentes entre depredador y presa (Pike et al. 2002, Pike et al. 2019). Además de esto, en las últimas décadas el calentamiento global ha afectado al ecosistema oceánico de Islandia (ver por ejemplo Valdimarsson et al. 2012), alterando la distribución y abundancia de numerosas presas que forman parte de la dieta de las ballenas (Stefansdottir et al. 2010, Víkingsson et al. 2014). Por tanto, conocer qué recursos explota cada una de las especies es esencial para poder predecir cómo se verán afectadas por los futuros cambios en el ecosistema.

2.2. Rorcual común en Islandia

De entre todas las especies de balaenoptéridos que ocupan las aguas de Islandia, el rorcual común es la que se suele avistar con mayor frecuencia (Pike et al. 2019). Es más abundante en la región oeste (stock “WI”, con algunos posibles individuos del stock colindante “EG”, Figura 3) que en la región este (stock “EI + F”, Figura 3), y durante las últimas décadas, se ha detectado un aumento en el número de rorcuales avistados en ambas regiones (Víkingsson et al. 2009, Pike et al. 2019). Aunque se ha especulado con un posible cambio en la distribución, que podría haber desplazado a los rorcuales hacia zonas más norteñas, la opción más aceptada es que en los últimos años se ha producido un aumento generalizado del rorcual común en el Atlántico Norte (Pike et al. 2019).

Como ocurre en el resto del Atlántico Norte, la mayoría de información que se tiene sobre los rorcuales de los stocks islandeses se basan en los datos recopilados durante los meses de verano. Los contenidos estomacales de rorcuales capturados en el oeste de Islandia muestran que su presa principal son los eufausiácedos, conocidos de forma genérica como krill. En concreto, casi toda su dieta se basa en la especie de krill predominante en la zona, *Meganyctiphanes norvegica* (Víkingsson 1997), al igual que

sucede en la mayoría de los stocks del Atlántico norte (Jönsgard 1966, Aguilar 1985, Aguilar and García-Vernet 2018). En otras zonas también consume ocasionalmente otras especies, como por ejemplo *Thysanoessa inermis* (Jönsgard 1996), aunque en Islandia parece que el consumo de *T. inermis* es marginal (Víkingsson 1997).

Durante las temporadas en las cuales la abundancia de eufausiácedos decae, el rorcual común también puede alimentarse de bancos de peces, y principalmente ha sido asociado a bancos de capelán (*Mallotus villosus*) y de arenque (*Clupea arengus*) en las costas noruegas (Christensen et al. 1992). En Islandia se ha identificado pescado en los estómagos de algunos individuos, y se infiere que en esta región el consumo de peces es ocasional y constituye menos de un 5% de la dieta (Víkingsson 1997, Arregui et al. 2018). Generalmente, el aumento de consumo de pescado parece producirse durante el otoño, la primavera y, posiblemente, el invierno (Christensen et al. 1992, Rita 2021).

A diferencia de la dieta, la conectividad con el resto de los stocks del Atlántico Norte no está bien definida. Las hipótesis sobre las zonas de reproducción consideradas por la IWC barajan distintos niveles de mezcla entre los stocks existentes en el Atlántico Norte (IWC 2009). La única premisa consistente en todas las hipótesis es que el stock de España (Sp) está formado por una única subpoblación, que se reproduciría en zonas distintas a las del resto de stocks del Atlántico Norte, incluidos los rorcuales de Islandia (IWC 2009). Este aislamiento entre los rorcuales de Islandia y España ha sido respaldado por análisis de isótopos (Vighi et al. 2016), morfológicos (Jover 1992) y de metales pesados (Sanpera et al. 1996), entre otros. Sin embargo, los análisis genéticos realizados hasta la fecha no han sido concluyentes (ver por ejemplo Berubé et al. 1998).

Las zonas de invernada y los patrones migratorios que siguen los rorcuales del stock islandés también son desconocidos. El marcaje satelital ha demostrado que algunos individuos que se alimentan en las Azores durante la primavera se desplazan posteriormente a las zonas de alimentación localizadas en el oeste de Islandia y el este de Groenlandia (Silva et al. 2013). Hasta la fecha, este es uno de los pocos corredores migratorios que se ha identificado en el Atlántico norte para esta especie, y relaciona claramente los individuos que ocupan las costas de Azores durante la primavera con los que veranean en las costas de Islandia. Además de esto, recientemente se han marcado satelitalmente individuos que veranean en las islas de Svalvard. Los registros muestras una dispersión considerable al llegar la migración otoñal: algunos individuos permanecieron en las islas Svalvard, mientras que otros realizaron migraciones latitudinales considerables. En concreto, se localizó una potencial área de reproducción

e invernada para la población noruega, situada en las costas de Portugal (Lydersen et al. 2020), zona asociada tradicionalmente al stock español (IWC 2009).

Este último estudio evidencia por tanto algunos puntos importantes. En primer lugar, es probable que algunos individuos se mantengan en altas latitudes durante el invierno, algo que también parece suceder en la población de Islandia (Gunnlaugsson and Víkingsson 2014). En segundo lugar, sugiere una ocupación secuencial de zonas asociadas tradicionalmente a otro stock, algo también sugerido en otros estudios (Silva et al. 2019, Gauffier et al. 2020). Todos estos resultados complican más aún si cabe un complejo escenario difícil de resolver, que necesita de herramientas y estrategias específicas para lograr responder cuestiones concretas.

3. Marcadores químicos. Los isótopos estables

3.1. Isótopos estables. Contexto general

Los isótopos son los distintos átomos de un mismo elemento químico, con un mismo número de protones pero diferente número de neutrones, lo que les confiere una masa distinta. En concreto, los isótopos estables son aquellos que tienen núcleos estables, por lo que no se desintegran con el paso del tiempo como ocurre con los isótopos radioactivos (Hoefs 2018).

La mayoría de los elementos con un interés biológico presentan dos (o más) isótopos estables, aunque en general uno de los isótopos es mucho más abundante que el resto de formas. La abundancia relativa de cada uno de los isótopos de un elemento varía ligeramente según algunos factores predecibles, y esto permite su aplicación en múltiples campos de estudio, incluido el campo de la ecología. En una muestra, esta abundancia relativa se cuantifica como la ratio del isótopo pesado respecto al ligero, dividido por la misma proporción cuantificada en una muestra estándar. Es decir:

$$\delta\,{}^{i}E_{\,\text{Muestra}} = \frac{R\,({}^{i}E\,/\,{}^{j}E)_{\,\text{Muestra}}}{R\,({}^{i}E\,/\,{}^{j}E)_{\,\text{Estándar}}} - 1$$

Donde $R({}^{i}E\,/\,{}^{j}E)$ son las ratios entre el isótopo pesado (i) y el isótopo ligero (j) del elemento E, tanto para la muestra que se analiza como para un estándar de referencia. Al resultado obtenido nos referiremos como valores delta y/o ratio de isótopos del elemento en cuestión (Coplen 2011).

En el campo de la ecología sin duda los isótopos más aplicados son los del nitrógeno ($\delta^{15}N$) y el carbono ($\delta^{13}C$), aunque en los últimos años el uso de los isótopos de azufre ($\delta^{34}S$), oxígeno ($\delta^{18}O$) e hidrógeno (δD) también ha aumentado notablemente. Al igual que otros compuestos químicos, los isótopos estables pueden ser medidos en múltiples organismos y tejidos, y son utilizados para inferir datos de la biología del animal, tales como la dieta o el hábitat (Boecklen et al. 2011, Ramos and González-Solís 2012).

La elección del tejido a analizar resulta de gran importancia durante el diseño experimental, ya que dependiendo de la tasa de recambio del tejido la información proporcionada por los isótopos será distinta. Por ejemplo, en tejidos de renovación rápida, como puede ser el plasma sanguíneo o el hígado, los isótopos estables proporcionan información sobre los días previos al muestreo (Ramos and González-Solís 2012, Vander Zanden et al. 2015). En el otro extremo encontramos tejidos con tasas de renovación muy lentas, como por ejemplo el hueso, que integra escalas temporales largas, del orden de años (Riofrío-Lazo and Aurioles-Gamboa 2013). Entre medio encontramos otros tejidos, como el músculo y la piel, que integran una escala temporal del orden de semanas / meses (Giménez et al. 2016, Busquets-Vass et al. 2017). Finalmente tenemos un último tipo de tejidos en los cuales no se produce recambio isotópico, ya que son biológicamente inertes y mantienen su composición química una vez han sido sintetizados. Dentro de esta categoría encontramos los tejidos queratinosos, como el pelo, las uñas y las barbas de ballena. En estos tejidos se produce también un crecimiento continuo y secuencial, por lo que guardarán un registro cronológico de los movimientos y los cambios de dieta del animal (Ramos and González-Solís 2012).

3.2. Isótopos de nitrógeno y carbono

La principal aplicación de los isótopos de nitrógeno se basa en su relación con el nivel trófico (Kelly 2000). La ratio de $^{15}N/^{14}N$ aumenta de manera predecible con el nivel trófico, debido principalmente a la excreción preferente del isótopo ligero a través de la orina (Peterson and Fry 1987). Este tipo de procesos, en los que una reacción enzimática selecciona como sustrato preferente a un isótopo concreto, dan lugar a un fraccionamiento isotópico, que en este caso concreto acabará generando una discriminación trófica entre depredador y presa. Esto supone que los valores $\delta^{15}N$ aumentan entre un 2 - 4‰ por cada nivel trófico, aunque el incremento puede variar según el organismo y el tejido analizado (Kelly 2000, Caut et al. 2008). En cetáceos se ha determinado la discriminación trófica para delfines mulares (ver por ejemplo Gimenez

et al. 2016), orcas (Caut et al. 2010), rorcual común (Borrell et al. 2012) y ballena azul (Vander Zander et al. 2014), aunque los tejidos analizados dependen del estudio. A nivel oceánico también encontramos importantes cambios geográficos, especialmente a nivel latitudinal. Por ejemplo, el Atlántico norte presenta valores más elevados de $\delta^{15}N$ en latitudes altas que en latitudes bajas (McMahon et al. 2013, Figura 6), por lo que los isótopos de nitrógeno servirían no solamente para estudiar la ecología trófica de los animales, sino también sus movimientos a escala oceánica.

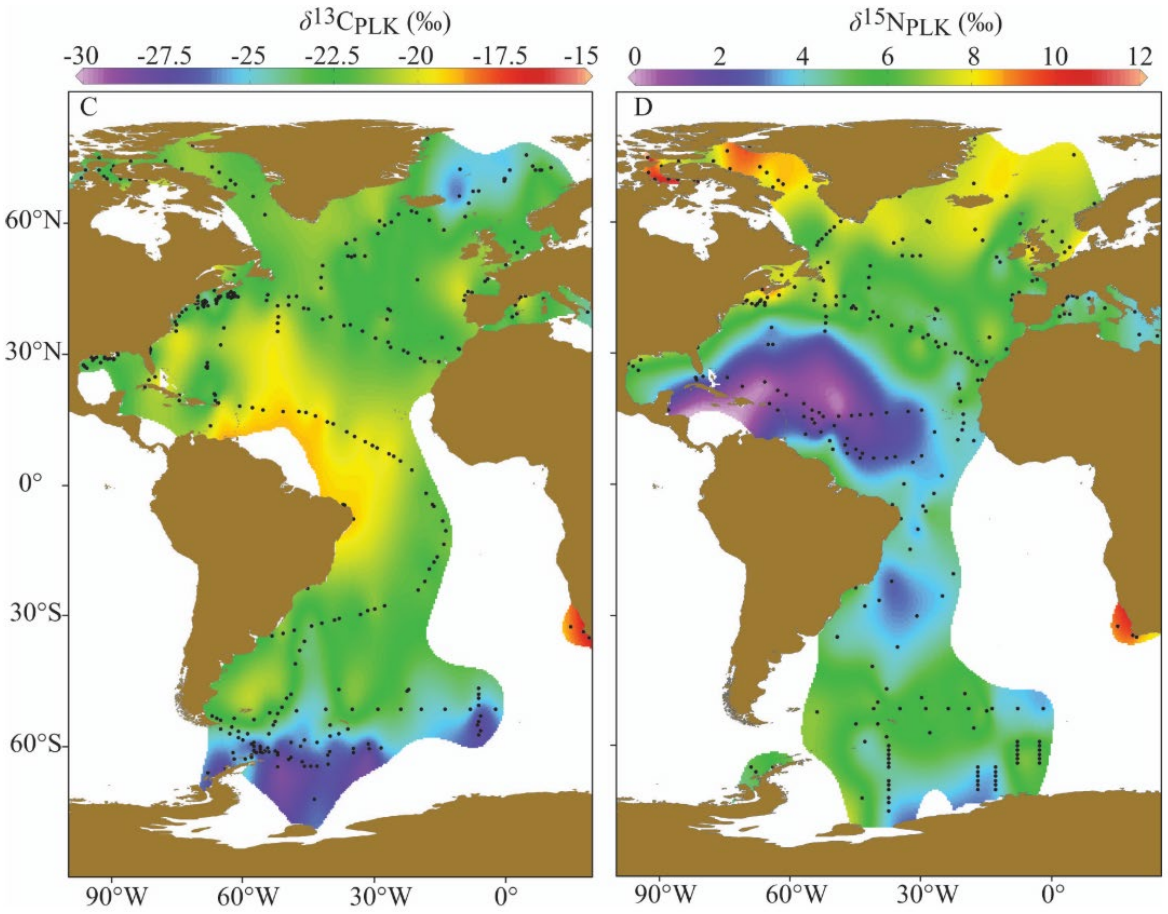


***Figura 6**: Variación espacial de los valores de $\delta^{13}C$ (izquierda) y $\delta^{15}N$ (derecha) analizados en fitoplancton del Atlántico. Nótese que en el Atlántico norte la variación de los valores de $\delta^{13}C$ es menos acusada que la variación de los valores de $\delta^{15}N$. Figura extraída de McMahon et al. (2013).*

En el caso del carbono, la discriminación trófica de la ratio de $^{13}C/^{12}C$ es mucho menor que la del nitrógeno, lo que provoca que no se emplee de forma directa para inferir el nivel trófico de los depredadores. Sin embargo, encontramos un fuerte fraccionamiento isotópico durante los procesos de fijación del carbono, que provoca que las plantas C3, las plantas C4 y el fitoplancton tengan valores de $\delta^{13}C$ distintos (Kelly 2000). Esta

característica permite diferenciar los ambientes costeros y/o bentónicos de los ambientes pelágicos y alejados de la costa, teniendo estos últimos valores más bajos de $\delta^{13}C$ (Hobson 1987, Hobson et al. 1994). Además de esto, al igual que con el valor de $\delta^{15}N$, también encontramos cambios latitudinales a nivel oceánico. En el Atlántico norte, esta variación latitudinal es menos acusada que para los valores de $\delta^{15}N$, y encontramos valores inferiores de $\delta^{13}C$ en las zonas de latitudes altas que en las latitudes bajas (McMahon et al. 2013, Figura 6).

Finalmente, destacar que otros factores fisiológicos, como las condiciones de ayuno, (Hobson et al. 1993, Polischuk et al. 2001), también podrían ocasionar cambios en la señal isotópica del nitrógeno y el carbono. Sin embargo, estos efectos no parecen estar tan claros en misticetos, en los que parece que ni las condiciones de ayuno ni el estado reproductivo afectan a de manera significativa la señal isotópica (Aguilar et al. 2014, Borrell et al. 2016)

3.3. Isótopos de azufre

En muchos casos, el análisis de otros isótopos estables puede complementar la información proporcionada por los isótopos de carbono y nitrógeno. Uno de los más utilizados actualmente son los isótopos de azufre.

A escala oceánica, los valores de $\delta^{34}S$ son relativamente homogéneos (Rees et al. 1978). Sin embargo, al igual que sucede con los isótopos de carbono, los valores $\delta^{34}S$ del fitoplancton son significativamente distintos a los valores de las plantas terrestres (Lott et al. 2003). Esto provoca que hasta ahora la mayoría de las aplicaciones del azufre en los mamíferos marinos se centran en diferenciar hábitats costeros de hábitats situados en mar abierto, en lugar de usarse para determinar movimientos a escala oceánica (Niño-Torres et al. 2006; MacAvoy et al. 2015; Borrell et al. 2021).

En general, se considera que la discriminación trófica para los isótopos de azufre es negligible (ver por ejemplo Krajcarz et al. 2019), por lo que de entrada su interpretación debería ser más sencilla que para el nitrógeno y el carbono. Sin embargo, recientemente se ha cuestionado si la discriminación trófica del azufre es tan negligible como se asume generalmente (McCutchan et al. 2003). Estas dudas se han visto reforzadas en ballenas, donde al analizarse barbas de distintos individuos de ballenas de Groenlandia (*Balaena mysticetus*) se encontró una correlación consistente entre los valores delta del nitrógeno y del azufre (Matthews and Ferguson 2015). Así pues, aunque el azufre es de entrada una herramienta prometedora para perfilar los resultados obtenidos con el nitrógeno y

el carbono, estos puntos podrían implicar una dificultad extra a la hora de interpretar sus resultados.

4. Marcadores moleculares. Metilación

4.1. Genética aplicada para el estudio del rorcual común

Los análisis genéticos han sido ampliamente utilizados para analizar muestras de mamíferos marinos. Los objetivos de estos estudios son muy diversos, y abarcan desde temas taxonómicos y de estructura poblacional (por ejemplo, Árnason et al. 2018), hasta temas relacionados con la adaptación al medio marino (ver por ejemplo Hyung-Soon et al. 2014). En el caso del rorcual común, la gran mayoría de los estudios se han centrado en tratar de resolver las incógnitas existentes respecto a su organización taxonómica y su estructura poblacional.

En general, los estudios genéticos han resultado útiles para resolver las relaciones existentes con especies próximas, más allá de las similitudes morfológicas (Árnason et al. 2018, McGowen et al. 2020). Sin embargo, los análisis filogenéticos focalizados en el rorcual común han generado más discusión, obteniendo resultados distintos según el número de muestras analizadas y abriendo el debate de si es necesaria una revisión taxonómica de la especie (Archer et al. 2013, Cabrera et al. 2019). Si a escala mundial ya se han obtenido resultados dispares, lograr determinar la estructura poblacional dentro de un solo océano ha resultado todavía más complicado. En general todas las muestras son tomadas en las zonas de alimentación, en las cuales podrían estarse agrupando rorcuales procedentes de distintas zonas de reproducción, dificultando aún más si cabe la interpretación de los resultados (Berubé et al. 1998).

En el Atlántico norte se han realizado multitud de estudios, utilizando diversos marcadores genéticos (revisado en Pampoulie and Danielsdottir 2013), y en general se ha visto que a nivel de secuencia de ADN no se aprecia una estructuración poblacional clara. Algunos estudios se han basado en secuenciar y analizar loci no neutrales, es decir, sujetos a una presión selectiva (Olsen et al. 2014), mientras que otros se han basado en loci neutrales, como ciertas regiones mitocondriales o microsatélites (Berubé et al. 1998). En todos los casos se obtuvieron bajos niveles de diferenciación genética entre los stocks del Atlántico norte, con excepción de la población Mediterránea (Palsboll et al. 2004, Berubé et al. 2006, Pampoulie and Danielsdottir 2013). Así pues, resulta evidente la necesidad de nuevas estrategias y marcadores moleculares para tratar de discernir las posibles diferencias existentes entre los stocks del Atlántico norte.

Finalmente, cabe destacar el uso de marcadores moleculares para determinar el grado de parentesco entre individuos. Esta metodología fue aplicada por primera vez en ballenas de Islandia por Skaug et al. (2006), obteniendo una serie de resultados preliminares a partir de los cuales se delimitaron a grandes rasgos las bases teóricas de este método. El sistema más empleado hasta la fecha es la puntuación LOD (LOD score), que se calcula a partir de la comparación por pares de distintos perfiles genéticos, generalmente generados a partir de la secuenciación de microsatélites. Dos individuos serán clasificados como emparentados cuando la puntuación LOD exceda de un valor crítico predefinido. Esta metodología ha sido aplicada con éxito para detectar relaciones de parentesco cercanas (padres – hijos, hermanos o medio hermanos, y primos) en rorcual aliblanco (Skaug et al. 2010, Tiedemann et al, 2012) y en rorcual común (Pampoulie et al. 2013).

4.2. Metilación del ADN

Entendemos como metilación de ADN a una modificación posterior a la replicación, en la cual un nucleótido adquiere un grupo metilo, que se unirá a través de un enlace covalente (Ahuja and Issa 2000). En vertebrados, la metilación ocurre principalmente en las citosinas de las secuencias citosina-fosfato-guanina (dinucleótidos CpG), y está vinculada a la represión transcripcional de los genes cercanos (Klose and Bird 2006). A nivel general, el genoma es pobre en CpG; sin embargo, esta escasez general contrasta con zonas altamente enriquecidas en este dinucleótido. Estas zonas, que reciben el nombre de islas de CpG (CGIs), suelen tener una longitud de centenares de pares de bases y un alto porcentaje de nucleótidos C y G. Se calcula que un elevado porcentaje de promotores de genes están asociados a islas CpG (Saxonov et al. 2006, Deaton and Bird 2011). En general, las CGIs no suelen estar metiladas, en contraste con el resto de las regiones genómicas. La metilación de las CGIs suele estar relacionada con el silenciamiento del promotor al cual se encuentran asociadas, aunque se cree que éste no es el factor desencadenante, sino que actúa como un bloqueador del estado de silenciamiento (Deaton and Bird 2011).

La metilación del ADN no es estática con el tiempo, viéndose modificada a lo largo del desarrollo (Smith and Meissner 2013) y con el envejecimiento. A nivel global, el envejecimiento suele estar asociado a una hipometilación general del genoma (Wilson et al. 1987, Ahuja and Issa 2000), mientras que en las CGIs se ha asociado el envejecimiento tanto a hipermetilación como a hipometilación, dependiendo de la región. Estas relaciones lineales entre edad y metilación han permitido desarrollar

metodologías para predecir la edad de los individuos, a partir del análisis de los niveles de metilación en zonas concretas del genoma (Goel et al. 2017).

El envejecimiento no es el único factor que altera los niveles de metilación a lo largo de la vida del individuo. Algunos factores ambientales, como por ejemplo cambios en la temperatura, salinidad, u otros factores estresantes, parecen inducir importantes modificaciones en la metilación del ADN, que en ciertos casos también han sido asociados con cambios en la expresión génica (Feil and Fraga 2012). Estas variaciones funcionales inducidas por el ambiente permitirían que una población exhibiera variabilidad fenotípica, a pesar de ser genéticamente homogénea (Flores et al. 2013). En principio, estas modificaciones no deberían ser heredables, ya que durante el desarrollo de la línea germinal se produce una reprogramación del metiloma a nivel global (Kota and Feil 2010), aunque algunas regiones del ADN parecen ser más resistentes a esta reprogramación (Feil and Fraga 2012).

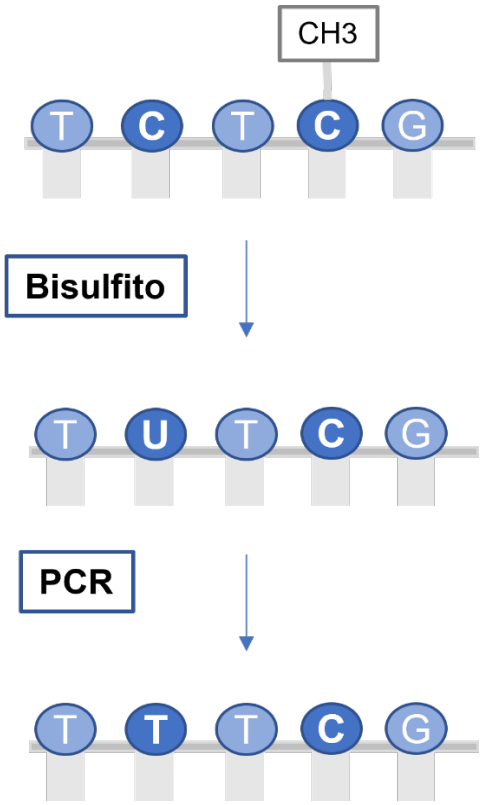


***Figura 7**: Esquematización del efecto del tratamiento del bisulfito en el ADN. En el producto final se tendrán principalmente 3 nucleótidos (T, G y A), ya que todas las citosinas no metiladas pasarán a convertirse en timinas.*

La mayoría de los métodos que permiten detectar si una determinada citosina se encuentra metilada están basados en la conversión con bisulfito (Figura 7). El bisulfito desamina las citosinas que no están metiladas, lo cual causa su conversión a uracilos, que tras una posterior amplificación mediante PCR pasarán a ser timinas.

Tras tratar el ADN con bisulfito, éste puede ser amplificado mediante PCR, para posteriormente secuenciarse ya sea a nivel de regiones concretas (Tost and Gut 2007) o a nivel de genoma (epigenoma) (por ejemplo, Boyle et al 2012, Olova et al. 2018). En el producto resultante, aquellas citosinas que no estaban metiladas serán timinas, mientras que las citosinas que si lo estaban seguirán manteniéndose como citosinas.

Este proceso permite la detección de citosinas metiladas con una resolución a nivel de nucleótido (Darst et al. 2010).

Teniendo en cuenta que las poblaciones celulares son heterogéneas, el resultado que se obtendrá será un porcentaje indicativo de la proporción de células de un mismo tejido que se encuentran metiladas en una determinada posición CpG.

4.3. Metilación del ADN para inferir la edad en el rorcual común

Hasta ahora, la mayoría de los estudios epigenéticos, incluidos los estudios que analizan los patrones de metilación de ADN, han estado realizados con muestras humanas y de animales modelo. Sin embargo, a lo largo de los últimos años, se han comenzado a aplicar algunas de estas técnicas moleculares para estudiar poblaciones salvajes. En el caso de la metilación, existen dos aplicaciones principales que han atraído el interés de la comunidad científica.

La primera aplicación es el uso de los niveles de metilación de citosinas concretas como predictores de la edad biológica, lo que se conoce como “reloj epigenético” (Horvath and Raj 2018). En humanos existen diferentes relojes epigenéticos, desarrollados a partir de muestras procedentes de tejidos de interés forense, como puede ser la sangre (Hannum et al. 2013), la piel (Grönniger et al. 2010) o múltiples tejidos (Horvath 2013, Levine et al. 2018). En especies salvajes, la edad es un parámetro crítico para el estudio de algunos parámetros poblacionales básicos, como pueden ser la longevidad o la maduración sexual; sin embargo, en la mayoría de los casos esta información es muy difícil de obtener (De Paoli-Iseppi et al. 2017). De forma reciente, se han desarrollado relojes epigenéticos para algunas especies salvajes, como pueden ser por ejemplo los chimpancés (Ito et al. 2018), aves marinas (De Paoli-Iseppi et al. 2018), o ballenas jorobadas (Polanowski et al. 2014) y belugas (Bors et al. 2021).

En el rorcual común, al igual que en otras ballenas, la edad se suele estimar realizando lecturas de las bandas de crecimiento que tienen en los tapones de cera del conducto auditivo (Lockyer 1984). Sin embargo, este tipo de muestras son difíciles de obtener, y requieren que el animal esté muerto. Otro tipo de técnicas se han tratado de aplicar para determinar la edad de las ballenas, incluyendo los análisis de la longitud de los telómeros (Olsen et al. 2014) y los análisis de ácidos grasos específicos (Hernan et al. 2009). Sin embargo, la resolución de estos predictores ha sido relativamente baja. Los análisis en los niveles de metilación podrían proporcionar una técnica útil para estos casos, pero resulta esencial encontrar marcadores óptimos que permitan una correcta predicción a partir de la secuenciación de un bajo número de citosinas, para que resulte económicamente viable su integración en los estudios poblacionales. En el año 2014, se identificaron exitosamente tres posiciones CpG cuyos niveles de metilación

correlacionaban con la edad en ballenas jorobadas, permitiendo la generación de un modelo predictivo con bajo error (Polanowski et al. 2014). Teniendo en cuenta su proximidad filogenética con el rorcual común, y su validación en múltiples poblaciones de ballena jorobada, la aplicación de estos tres biomarcadores resulta una opción prometedora para inferir la edad en el resto de los rorcuales.

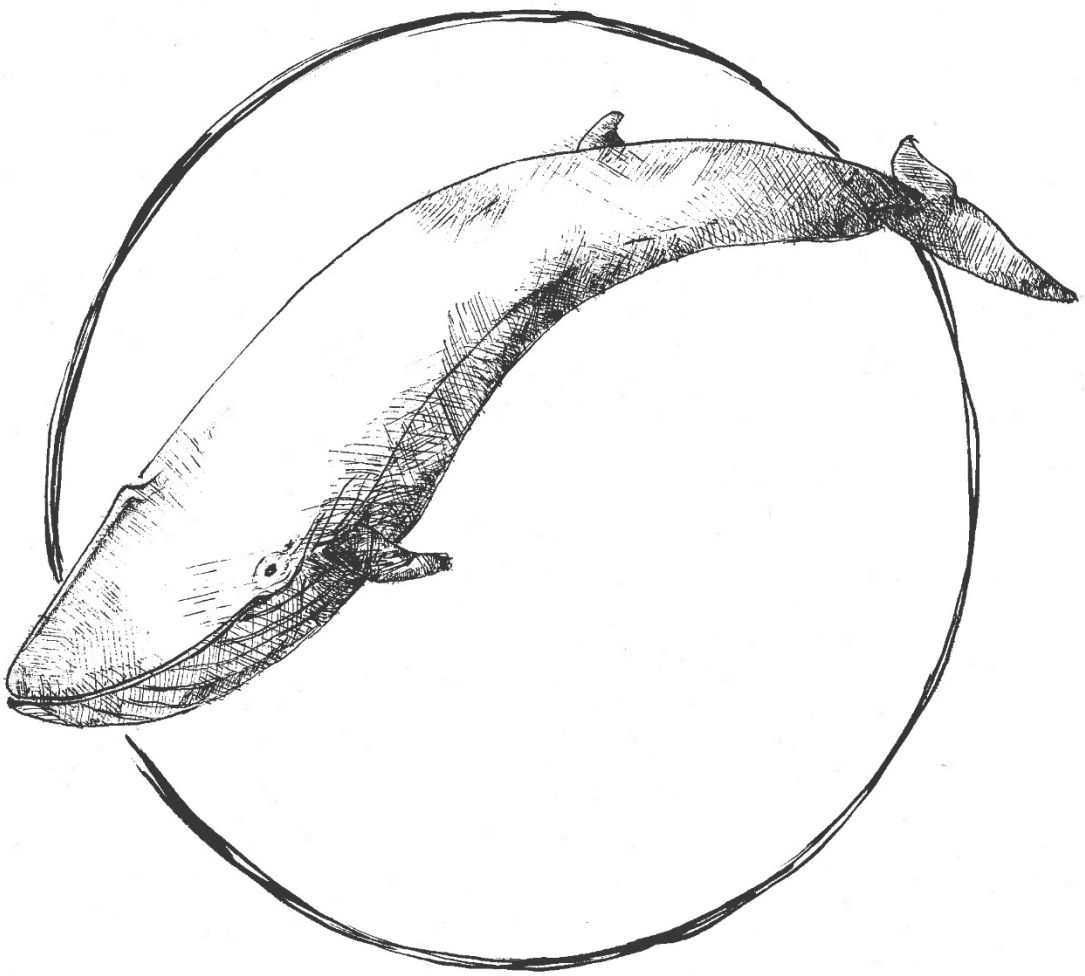
4.4. Metilación del ADN y adaptación al ambiente en el rorcual común

La segunda aplicación con un claro potencial para el estudio de poblaciones salvajes es el papel que tiene la metilación del ADN en el proceso de adaptación ambiental y su potencial a nivel evolutivo (Flores et al. 2013, Verhoeven et al. 2016). Al igual que en el caso anterior, se trata de un campo novedoso que despegó a raíz de la relación existente entre patrones de metilación anómalos y el desarrollo de cáncer (Ahuja and Issa 2000). En humanos, se ha encontrado relación entre factores ambientales y/o factores estresantes con patrones característicos en la metilación del ADN (por ejemplo, Grönniger et al. 2010, Jacobsen et al. 2012, McGuiness et al. 2012), que en algunos casos también podrían asociarse a un envejecimiento prematuro a nivel de metilación del ADN (Dhingra et al. 2018).

Dentro del campo de la ecología y la evolución, se ha visto que la plasticidad fenotípica que se observa en algunos procesos migratorios se asocia con regiones diferencialmente metiladas entre poblaciones residentes y migratorias de distintas especies de peces, incluso en ausencia de diferenciación genética (Baerwald et al. 2016, Whitaker et al. 2018, Merlin and Liedvogel 2019). Así mismo, otro tipo de factores, como pueden ser cambios de temperatura (Weyrich et al. 2016), o cambios en el acceso a los recursos a raíz de la urbanización (Lea et al. 2016, McNew et al. 2017, Watson et al. 2021), también producen diferencias en el epigenoma. Algunos de estos cambios parece que pueden ser transmitidos entre generaciones, implicando que el ambiente al cual se exponen los padres podría llegar a influenciar significativamente en la descendencia (Weyrich et al. 2016, Berbel-Filho et al. 2020).

A pesar de las evidencias existentes a raíz del uso de marcadores químicos, los análisis genéticos llevados a cabo hasta la fecha no han sido capaces de detectar una estructura poblacional del rorcual común del Atlántico Norte (Pampoulie and Danielsdottir 2013, Introducción 4.1). De todos los stocks existentes en el Atlántico Norte, el stock español parece ser el más aislado de todos ellos, y diversos estudios evidencian el aislamiento existente entre el stock de Islandia y el stock de España (Figura 4, Introducción 2.2.). Así pues, la metilación del ADN podría ser un buen marcador molecular para este caso,

ya que podría permitir la identificación de diferencias adaptativas entre poblaciones, a pesar de que sean móviles y presenten un flujo genético considerable (ver por ejemplo Meröndun et al. 2019).



OBJETIVOS

OBJETIVOS

El objetivo global de esta tesis es **ampliar el conocimiento sobre la biología y ecología del rorcual común, con foco principal en la población de rorcual común de Islandia**. Dadas las numerosas incógnitas que siguen existiendo alrededor de esta especie, se establecen una serie de objetivos concretos que serán respondidos en los distintos capítulos.

La presente tesis se divide en dos bloques principales. En el primer bloque, se aplicarán análisis de isótopos estables para responder preguntas principalmente relacionadas con la ecología trófica de la población de rorcual común de Islandia. Este bloque incluye los Capítulos 1 y 2.

En el segundo bloque, se utilizarán los análisis de isótopos estables junto con los análisis de la metilación del ADN para analizar principalmente la estructura migratoria y poblacional del rorcual común de Islandia, comparándola en algunos estudios con la población de rorcual común de España. En este bloque también se analizará el uso de la metilación de ADN para determinar la edad de los rorcuales comunes. Este bloque incluye los Capítulos 3, 4 y 5.

Los objetivos concretos establecidos en la presente tesis son:

a. Determinar la repartición de los recursos ecológicos entre las especies de misticetos que se alimentan en aguas islandesas.

En el **Capítulo 1**, se analizarán muestras de pieles de las cinco especies de misticetos (ballena azul, rorcual común, ballena jorobada, rorcual aliblanco y rorcual boreal), que se alimentan en aguas islandesas. Se determinarán los valores isotópicos del nitrógeno, carbono y azufre para inferir la dieta de cada especie y la posible competencia entre ellas.

b. Explorar el uso de las barbas de ballena para estudiar los potenciales movimientos migratorios y cambios en la dieta, analizando el efecto que puede tener el tamaño de la barba y su posición en la boca del animal en los resultados obtenidos.

En la primera parte del **Capítulo 2,** se examinará si los isótopos estables analizados en muestras de barbas procedentes de una misma ballena nos proporcionan la misma información, indistintamente de su tamaño y posición dentro de la boca del animal. Este

estudio preliminar es de gran importancia de cara a los estudios posteriores en los cuales se utiliza esta metodología, y en los que no siempre se sabe la posición que ocupaba la barba analizada.

c. Explorar la aplicabilidad de los isótopos de azufre en el estudio de los movimientos a escala oceánica del rorcual común

Como se ha expuesto en la Introducción, los isótopos de azufre han sido utilizados principalmente para diferenciar comportamientos costeros de comportamientos pelágicos. Sin embargo, cuando han sido aplicados para analizar barbas de ballenas, han surgido dudas durante su interpretación. Nuevamente en el **Capítulo 2**, se analizarán los isótopos de azufre (junto con isótopos de nitrógeno y carbono) para estudiar su potencial aplicación a escala oceánica, concretamente para el estudio de las migraciones y los cambios de dieta del rorcual común.

d. Determinar si existe algún tipo de estructuración en las migraciones del rorcual común de la población islandesa.

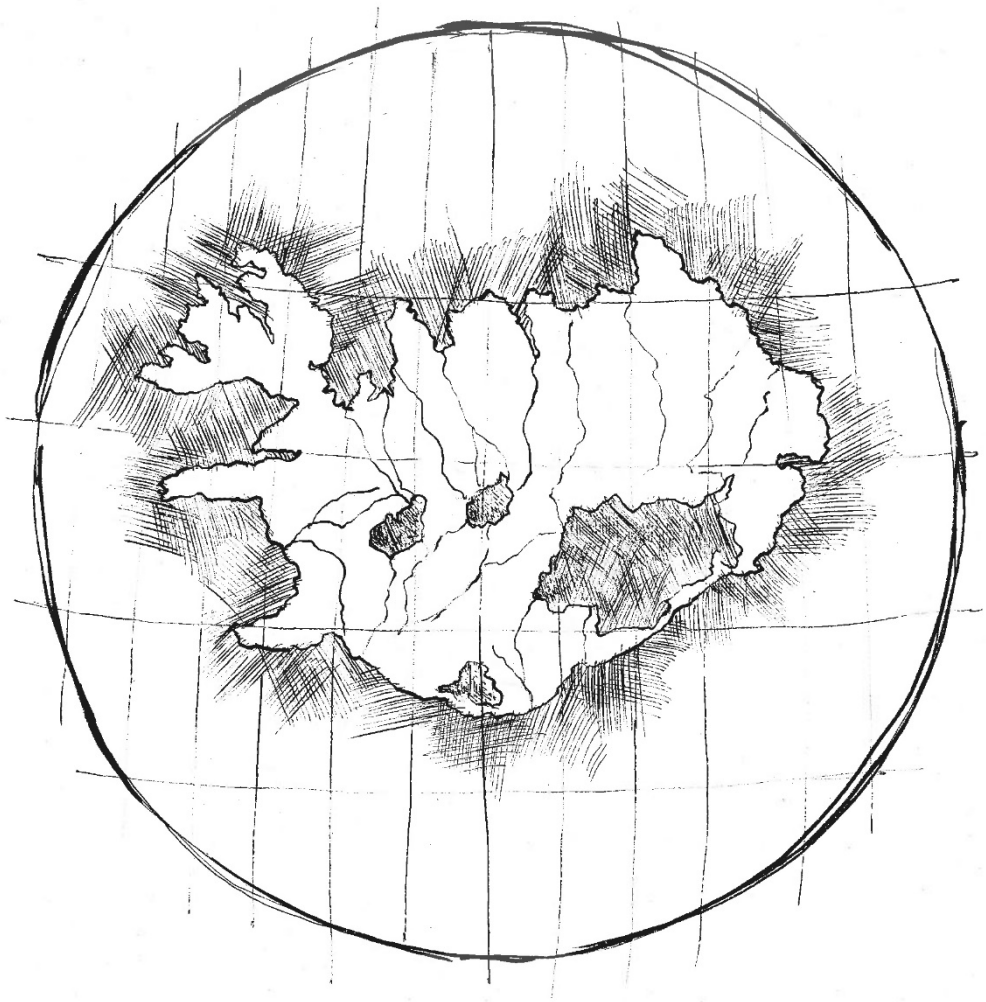
Las migraciones y el comportamiento del rorcual común fuera de las zonas de alimentación son ampliamente desconocidas. En el **Capítulo 3**, a través de los análisis de isótopos del nitrógeno en barbas, se comprobará si existe estructura en las migraciones efectuadas por los individuos de la población islandesa. Además, se realizarán análisis genéticos para determinar el grado de parentesco de los individuos analizados.

e. Comprobar la efectividad de la metilación en la determinación de la edad de los rorcuales comunes

Determinar la edad de los individuos es un paso esencial para poder comprender la estructura poblacional. En el **Capítulo 4**, se realizará un modelo predictor de la edad para el rorcual común a partir de marcadores epigenéticos. Así mismo, se estudiará el posible efecto del ambiente en estos predictores.

f. Determinar la existencia de diferencias epigenéticas entre dos poblaciones de rorcual común genéticamente homogéneas.

En el **Capítulo 5** se estudiarán las diferencias epigenéticas a nivel genómico que existen entre la población de Islandia y la población de España. A pesar de que existen multitud de evidencias sobre su aislamiento, hasta la fecha los marcadores moleculares no han permitido una correcta discriminación.



INFORME DE LAS DIRECTORAS

INFORME DE LAS DIRECTORAS

Las directoras Dra. Assumpció Borrell y Dra. Marta Riutort certifican que la presente tesis doctoral, titulada "Aplicación de técnicas moleculares y químicas al estudio de la dieta, migración y estructura poblacional del rorcual común de Islandia", ha sido llevada a cabo por Raquel García Vernet. A continuación, se detalla la contribución que ha realizado la doctoranda en cada uno de los seis artículos que componen su tesis, así como las revistas en las que se han publicado y su factor de impacto.

Capítulo 1 "Ecological niche partitioning between baleen whales inhabiting Icelandic waters". 2021. **García-Vernet R.**, Borrell A., Víkingsson G., Halldórsson S.D. and Aguilar A. Progress in Oceanography, 199: 102690.

Contribución de la doctoranda: Contribución al proceso de diseño experimental. Realización de los análisis de laboratorio, los análisis estadísticos, participación en la interpretación de resultados, redacción del manuscrito original.

El artículo está publicado en la revista *Progress in Oceanography*, que tiene un Factor de Impacto de 4.080 (2021). La revista se sitúa en la posición 4/64 del área de "Oceanografía" – en el percentil 92.97 (1er decil)

Capítulo 2 "Are stable isotope ratios and oscillations consistent in all baleen plates along the filtering apparatus? Validation of an increasingly used methodology". 2018. **García-Vernet R.**, Sant P., Víkingsson G., Borrell A. and Aguilar A. Rapid Communications in Mass Spectrometry, 32(15): 1257-1262.

Contribución de la doctoranda: Contribución al proceso de diseño experimental. Supervisión de los análisis de laboratorio, realización de los análisis estadísticos, participación en la interpretación de resultados, redacción del manuscrito original.

El artículo está publicado en la revista *Rapid Communications in Mass Spectrometry*, con un Factor de Impacto de 2.045 (2018). En el año 2018, la revista se situaba en la posición 20/41 del área de "Espectroscopía", en el percentil 56.92 (2º cuartil)

Capítulo 2 “Sulfur stable isotope ratios provide further insight into movements of the fin whale, an oceanic long-range migrant”. (in press). **García-Vernet R.**, Aguilar A., Zafra J., Víkingsson G., Halldórsson S.D. and Borrell A. Marine Ecology Progress Series.

Contribución de la doctoranda: Contribución al proceso de diseño experimental. Realización de parte de los análisis de laboratorio. Realización de todos los análisis estadísticos, participación en la interpretación de resultados, redacción del manuscrito original.

La revista Marine Ecology Progress Series tiene un factor de impacto de 2.824 (2020). Se sitúa en la posición 24/110 del área de “Biología marina y de agua dulce” en el percentil 78.65 (1º cuartil)

Capítulo 4 “CpG methylation frequency of TET2, GRIA2, and CDKN2A genes in the North Atlantic fin whale varies with age and between populations”. 2021. **García-Vernet R.**, Martín B., Peinado M.A., Víkingsson G., Riutort M. and Aguilar A. Marine Mammal Science 37(4): 1230-44.

Contribución de la doctoranda: Contribución al proceso de diseño experimental. Realización de los análisis de laboratorio, los análisis estadísticos, participación en la interpretación de resultados, redacción del manuscrito original.

El artículo está publicado en la revista *Marine Mammals Science*, con un Factor de Impacto de 2.090 (2020). Se situa en la posición 52/174 del área de “Zoología”, en el percentil 70.40 (2º cuartil)

Finalmente, en la presente tesis también se adjuntan dos manuscritos originales, que serán ambos enviados a revistas internacionales e indexadas. Estos artículos son:

Capítulo 3 “Order within chaos: Genetically unrelated fin whales may migrate in dyads”. **García-Vernet R.**, Rita D., Berubé M., Elgueta-Serra J., Pascual-Guasch M., Víkingsson G., Borrell A., Aguilar A.

Contribución de la doctoranda: Contribución al proceso de diseño experimental. Realización de parte de los análisis de laboratorio, realización de todos los análisis estadísticos, participación en la interpretación de resultados, redacción del manuscrito original.

El artículo ha sido revisado por todos los autores, y se planea enviarlo a la revista *Frontiers in Marine Science*, con un factor de impacto de 4.912. Se sitúa en la posición 6/110 del área de "Biología marina y de agua dulce" (1er cuartil).

Capítulo 5 "A preliminary analysis of fin whale methylome shows migration-related epigenetic differences between two north Atlantic populations". **García-Vernet R.**, Cuesvas C., Aguilar A., Borrell A., Riutort M.

Contribución de la doctoranda: Contribución al proceso de diseño experimental. Realización de los análisis de laboratorio, realización de los análisis bioinformáticos y estadísticos, participación en la interpretación de resultados, redacción del manuscrito original.

El artículo se encuentra en preparación. Se planea enviar a la revista Epigenetics, con un factor de impacto de 4.528. Se sitúa en la posición 52/176 del área de "Genética y Herencia genética" (2º cuartil).

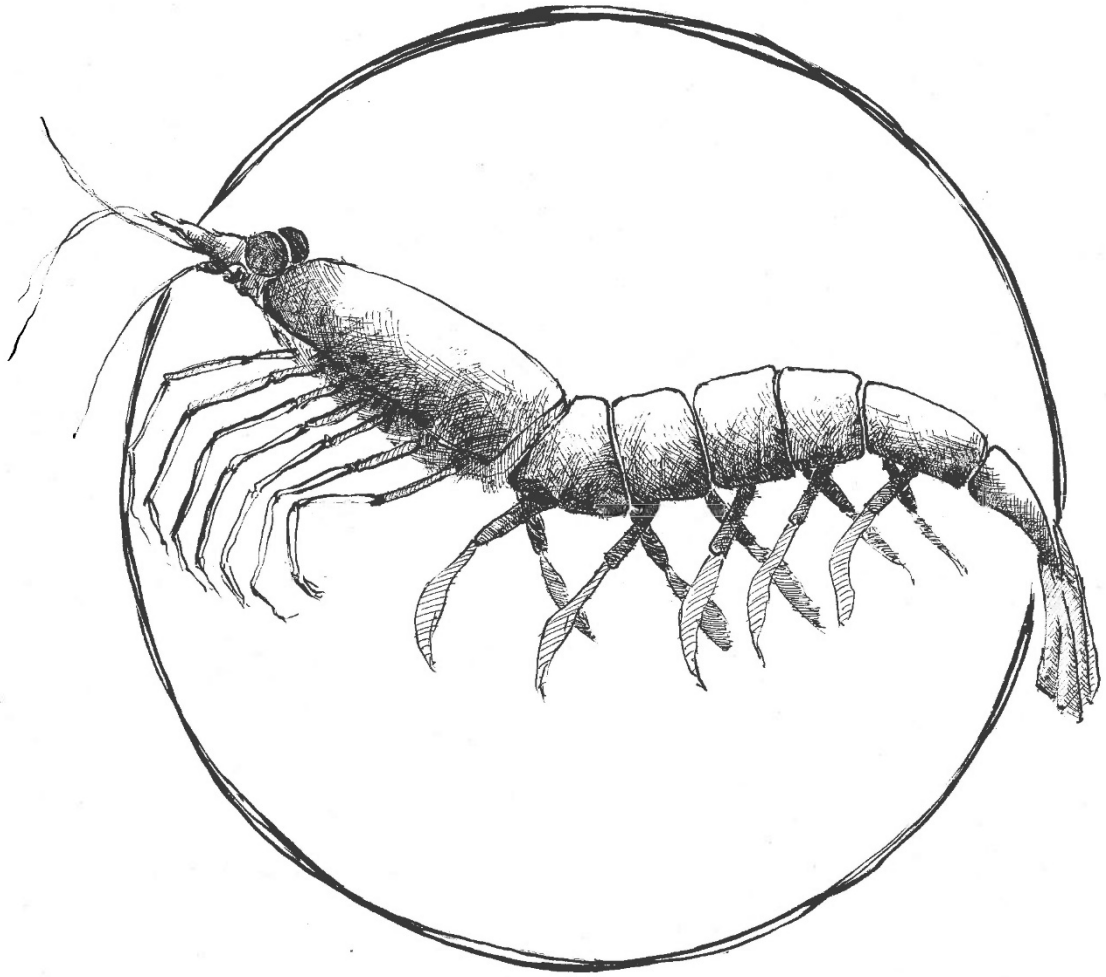
Barcelona, febrero de 2022,

Dra. Assumpció Borrell Thió

Dra. Marta Riutort León

Departamento de Biología Evolutiva,
Ecología y Ciencias Ambientales

Departamento de Genética,
Microbiología y Estadística



CAPÍTULO 1

Ecological niche partitioning between baleen whales inhabiting Icelandic waters

Autores: **Raquel García-Vernet**[a], Asunción Borrell[a], Gísli Víkingsson[b], Sverrir D. Halldórsson[b], Alex Aguilar[a]

[a] *Departamento de Biología Evolutiva, Ecología i Ciencias Ambientales, y IRBio, Facultad de Biología, Universidad de Barcelona*

[b] *Marine and Freshwater Research Institute, Fornubúðum 5, 220 Hafnarfjörður, Iceland*

The highly productive waters off Iceland are an important feeding ground for baleen whales. Five balaenopterid species coexist there during the summer feeding season: the blue whale, the fin whale, the sei whale, the humpback whale and the common minke whale. For capital breeders such as baleen whales, niche partitioning and reduced interspecific competition during their stay in the feeding grounds may be critical for the completion of their annual cycles and the long-term stability of populations. Coexistence often entails spatio-temporal or trophic segregation to avoid competitive exclusion.

With the aim of studying how these species share habitat and trophic resources, we analyzed the δ13C, δ15N and δ34S values in skin samples. Bayesian stable isotope mixing models to calculate compositional mixture of food sources showed that most species segregate by consuming different prey. Segregation was further enhanced by some degree of spatio-temporal exclusion.

Overall, clear ecological niche partitioning was apparent between all species except between blue and fin whales. All the species consumed krill and, except for the common minke whale, this was the dominant prey. Among baleen whales, common minke whales and humpback whales were the major predators of sand eel, capelin and herring. In humpback whales, a strong reliance on krill may explain the apparently low rates of local entanglement in fishing nets as compared to other areas.

Except for the blue whale, all species have shown evidence of adapting to shifts in prey availability and thus suggested capacity to cope with variability. However, in a scenario of increasing environmental variability associated to global warming, the overlap between ecological niches may have to decrease to allow long-term coexistence.

Progress in Oceanography 199 (2021) 102690

Contents lists available at ScienceDirect

Progress in Oceanography

journal homepage: www.elsevier.com/locate/pocean

Ecological niche partitioning between baleen whales inhabiting Icelandic waters

Raquel García-Vernet [a], Asunción Borrell [a], Gísli Víkingsson [b], Sverrir D. Halldórsson [b], Alex Aguilar [a,*]

[a] Department of Evolutionary Biology, Ecology and Environmental Sciences, and IRBio, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain
[b] Marine and Freshwater Research Institute, Fornubúðum 5, 220 Hafnarfjörður, Iceland

ARTICLE INFO

Keywords:
Stable isotopes
Trophic niche
Diet
Niche partitioning
Cetaceans
Balaenopteridae

ABSTRACT

The highly productive waters off Iceland are an important feeding ground for baleen whales. Five balaenopterid species coexist there during the summer feeding season: the blue whale, the fin whale, the sei whale, the humpback whale and the common minke whale. For capital breeders such as baleen whales, niche partitioning and reduced interspecific competition during their stay in the feeding grounds may be critical for the completion of their annual cycles and the long-term stability of populations. Coexistence often entails spatio-temporal or trophic segregation to avoid competitive exclusion. With the aim of studying how these species share habitat and trophic resources, we analyzed the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in skin samples. Bayesian stable isotope mixing models to calculate compositional mixture of food sources showed that most species segregate by consuming different prey. Segregation was further enhanced by some degree of spatio-temporal exclusion. Overall, clear ecological niche partitioning was apparent between all species except between blue and fin whales. All the species consumed krill and, except for the common minke whale, this was the dominant prey. Among baleen whales, common minke whales and humpback whales were the major predators of sand eel, capelin and herring. In humpback whales, a strong reliance on krill may explain the apparently low rates of local entanglement in fishing nets as compared to other areas. Except for the blue whale, all species have shown evidence of adapting to shifts in prey availability and thus suggested capacity to cope with variability. However, in a scenario of increasing environmental variability associated to global warming, the overlap between ecological niches may have to decrease to allow long-term coexistence.

1. Introduction

The ecological niche of a species can be understood as a multidimensional volume whose axes represent environmental and trophic variables and in which every point corresponds to a state of the environment which permits the survival of that species (Hutchinson 1957). Overlap between niches of species that co-exist in a given ecosystem should be necessarily limited to avoid an excessive competition (MacArthur and Levins 1967, Schoener 1983) that may end up with the exclusion of one of the competing species (Hardin 1960, Pianka 1974).

Marine organisms show consistently higher frequencies of competition than terrestrial ones, as do large-sized organisms compared to smaller ones (Connell 1983). Baleen whales or mysticetes are marine organisms and include the largest animals on Earth. They are filter feeders and all of them, except the gray whale (*Eschrichtius robustus*), which mostly preys on benthic crustaceans, exploit prey that thrive in the water column, a fact that inevitably involves some degree of interspecific competition (Mori and Butterworth 2006). After centuries of exploitation, once protection came into force the recovery of the various species and populations has been heterogeneous (Best 1993, Clapham et al., 2008, Thomas et al., 2016) and this has triggered debate on the potential effect of interspecific competition for food and its interplay with the long-term demographic trajectory of populations (Clapham and Brownell 1996, Friedlaender et al., 2009, Konishi et al., 2008). Similar debate has been raised with regards to the competition of baleen whales with commercial fisheries, and this has led in some instances to the proposal that a reduction in whale biomass may translate into a corresponding increase in the species consumed by whales which would then

* Corresponding author at: Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Av. Diagonal, 643, 08028 Barcelona, Spain.
E-mail address: aaguilar@ub.edu (A. Aguilar).

https://doi.org/10.1016/j.pocean.2021.102690
Received 3 March 2021; Received in revised form 2 September 2021; Accepted 6 October 2021
Available online 15 October 2021

become available to fisheries (Schweder et al., 2000). This has become an argument to support whaling independently of their direct economic exploitative benefits (Morissette et al., 2012, Ruzicka et al., 2013).

All this demands for a more precise delineation of the ecological niches of baleen whales. Although both diet and ecological niche are dynamic and may vary ontogenetically and between seasons and years responding to environmental shifts (Gómez-Campos et al., 2011, Fleming et al., 2016), niche delineation shall permit a better understanding of the place of baleen whales in ecosystems. In particular, it should allow an accurate integration of these organisms as functional groups in trophic web models (Jusufovski et al., 2019) and in this way contribute to the in-depth assessment of potential competition between baleen whales and fisheries (Stefánsson et al 1997). Moreover, projections on the impact that climate change may have on baleen whales point to population declines as a consequence of reduced prey from warming and increasing interspecific competition between whale species or between whales and fisheries (Tulloch et al., 2019; Bogstad et al., 2015). In this scenario, the need for reliable information on diet composition and habitat use is particularly urgent in polar ecosystems, where both the ecosystems and their marine mammal populations are expected to experience substantial environmental pressures caused by the foreseen climate shifts (Huntington 2009, Moore et al 2019).

Traditional methods for determining feeding ecology, such as fecal analysis, stomach contents analyses, or observations of feeding behavior provide only information of the most recently consumed prey, and thus yield an incomplete picture of overall diet, and can be biased by differences in the digestibility of prey and in the easiness of species-identification of body parts (Bowen and Iverson 2013, Trites and Spitz 2018). The stable isotope composition of the tissues of an individual contains the label of both the assimilated diet and the environment in which the individual lives. Consequently, tissue stable isotope analysis has become a useful complementary tool to investigate the place of wild animals in their ecosystems (Kelly 2000, Newsome et al., 2010). The use of tissues with relatively high turnover, such as skin with a turnover of a few months (Busquets-Vass et al., 2017), can be used to draw stable isotope niches that reflect the bionomic elements sustaining organisms in a given area and season, and thus infer the ecological niches of cohabiting species (Newsome et al., 2007, Pinela et al., 2010, Gavrilchuk et al., 2014). Although most studies of this nature rely on the application of stable isotope biplots (usually $\delta^{13}C$ and $\delta^{15}N$), the strength of the assessment increases if the stable isotopes of further elements (e.g. $\delta^{34}S$) are incorporated into the analysis because the various elements contribute different information (Connolly et al., 2004; Swanson et al., 2015). Thus, while all isotope ratios reflect baseline levels (McMahon et al., 2013), $\delta^{15}N$ and, to a lesser extent, $\delta^{13}C$ values vary with trophic level. $\delta^{13}C$ values also provide general information about spatial distribution because they mirror the primary C sources and thus tend to be higher in coastal or benthic primary producers than in offshore or pelagic primary producers. Finally, $\delta^{34}S$ values decrease with freshwater inputs and therefore also vary with proximity to coast (e.g. Borrell et al., 2021).

Iceland (63–66°N) is located just at the Arctic Circle, at the juncture of Arctic and North Atlantic oceans, and the high productivity of its waters makes them an important foraging area for baleen whales during the summer (Sigurjónsson, 1995). The most common species there are the common minke whale (*Balaenoptera acutorostrata*), the fin whale (*Balaenoptera physalus*), the sei whale (*Balaenoptera borealis*), the humpback whale (*Megaptera novaeangliae*) and the blue whale (*Balaenoptera musculus*). Although a small part of the population of some or all of these species may remain around Iceland throughout the year, the largest component of all species undertakes annual migrations and alternate low-latitude breeding grounds in winter with the high-latitude Icelandic feeding grounds in summer (Sigurjónsson and Víkingsson 1997, Magnusdottir and Lim 2019, Lydersen et al., 2020). The coexistence of these five species with similar ecological requirements in Iceland is intriguing, and leads to questioning how they share the available resources to avoid competitive exclusion. In-depth studies on diet have been conducted on the two species that have been exploited commercially until recent times, the common minke whale (Sigurjónsson et al., 2000, Víkingsson et al., 2014) and the fin whale (Víkingsson 1997), but information on the diet composition and ecological niche of the other species is scant or absent. In addition, in the last decades the effects of global warming have become apparent in the oceanic ecosystem off Iceland (Sarafanov et al., 2007, Pálsson et al., 2012b) and this has led to changes in the composition, distribution and abundance of numerous species that constitute baleen whale prey (Stefansdottir et al., 2010, Silva et al., 2014, Víkingsson et al., 2014; Gíslason et al., 2009; Asthorsson et al., 2012). These changes necessitate continuous re-evaluation of feeding and habitat-use parameters.

Here we present results of a study conducted through the stable isotope analyses of three elements (nitrogen, carbon and sulfur) in skin samples from the five baleen whale species inhabiting Icelandic waters. While our first objective was to investigate the diet composition and potential overlap in trophic niches of these species in Iceland, the study also allowed to gain some perspective on their interaction with the local fisheries as well as on plausible trends in their ecology in a scenario of global warming.

2. Material and methods

2.1. Sample collection and preparation

Details about the sampling of skin from the baleen whale species are shown in Table S1. The skin samples from humpback and blue whales were collected using biopsy darts shot to free-ranging individuals during the summer, while for sei and fin whales they were obtained from individuals caught off West Iceland, and for common minke whales from individuals taken by different boats around Iceland, all of them also collected during the summer. In all cases, skin was obtained from the dorsal region of the central portion of the body trunk. Although some variation may exist in the precise body location sampled, this is not expected to affect the study as skin has been shown to be a homogeneous tissue with regards to its stable isotope composition (Borrell et al., 2018b). Krill samples were obtained from fresh stomach contents from fin whales caught off W Iceland in 2018 and flensed at the Hvalur H/F station. All samples were preserved at −20 °C. The stable isotope values from other prey consumed by the whales were obtained from the literature.

Prior to analyses, the samples were dried for 24 h at 50 °C, and ground to powder using a mortar and pestle. To avoid the decrease of $\delta^{13}C$ values produced by lipids (DeNiro and Epstein 1977), the lipidic fraction was removed by soaking the skin samples in a chloroform/methanol (2:1) solution following the Folch method (Folch et al., 1957) and shaking them with a rotator for 24 h. This process was sequentially repeated three times, and samples were dried before analysis.

2.2. Stable isotope analyses

For carbon and nitrogen analyses, powered samples of approximately 0.3 mg of skin and 1 mg of krill were weighed into tin capsules. Samples were loaded and combusted at 1000 °C and analyzed using a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Flash 1112 elemental analyzer; CE Elantech, Lakewood, NJ, USA), coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (both from ThermoFinnigan, Bremen, Germany). For sulfur analyses, powered samples of approximately 2 mg of skin were weighed into tin capsules. Samples were loaded and combusted at 1030 °C and analyzed with an Elemental Analyzer (Carlo Erba 1108) coupled to a Delta Plus XP isotope ratio mass spectrometer via a ConFlow III interface (both from Thermofisher).

The analytical results are presented according to the delta (δ) notation, where the relative variations of stable isotope ratios are expressed

in parts-per-thousand (‰) compared to predefined standards:

$$\delta X = [(R\ sample/R\ standard) - 1] * 1000$$

where X is ^{13}C, ^{15}N or ^{34}S, and R sample and R standard are the heavy-to-light isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$) in the sample and in the reference standards, respectively. These standards are the Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for ^{13}C, atmospheric nitrogen (air) for ^{15}N, and Vienna Canyon Diablo Troilite (V-CDT) for ^{34}S. The accuracy of measurements for $\delta^{13}C$, $\delta^{15}N$ and ^{34}S were 0.1, 0.3 and 0.2 ‰, respectively.

For $\delta^{13}C$ and $\delta^{14}N$, international isotope secondary standards of known isotope ratios in relation to V-PDB and air, respectively, were used. These were: polyethylene (IAEA-CH-7; $\delta^{13}C$ = -31.8‰), sucrose (IAEA-CH_6; $\delta^{13}C = -10.4$‰), ammonium sulfate (IAEA N1; $\delta^{15}N = +0.4$‰ and IAEA N2; $\delta^{15}N = +20.3$‰), potassium nitrate (USGS 34; $\delta^{15}N = -1.7$‰), L-glutamic acid (USGS 40; $\delta^{15}N = -4.6$‰; $\delta^{13}C = -26.2$‰) and caffeine (IAEA 600; $\delta^{15}N = 1.0$‰; $\delta^{13}C = -27.7$‰). For $\delta^{34}S$, secondary standards of known isotope ratios in relation to V-CDT were: barium sulfate (IAEA SO-6; $\delta^{34}S = -34.1$‰ and IAEA SO-5; $\delta^{34}S = +0.5$‰) and YCEM ($\delta^{34}S = +12.8$‰).

The reference materials used for the analysis were obtained from the International Atomic Energy Agency (IAEA). The analyses were carried out in the Centres Científics i Tecnològics of the University of Barcelona (CCiT-UB).

2.3. Statistical analyses

Because of the occurrence of the Suess effect, which is a significant decrease of ^{13}C in atmospheric CO_2 caused by the burning of fossil fuels (Keeling 1979), before conducting any statistical analysis the $\delta^{13}C$ values from both the baleen whale samples and the prey samples were converted to values corresponding to 2013 by considering a decrease of 0.027 ‰ yr^{-1} (Borrell et al., 2018a).

Data were tested for normality (Shapiro–Wilk test) and homoscedasticity (Bartlett test), and means and standard deviations were calculated for each baleen whale species. A Kruskal-Wallis test was performed for each stable isotope ratio to look for significant differences between species, followed by a post-hoc test (Dunn Test) adjusted with the Holm method (Ogle et al., 2020). Bayesian mixing models were applied to stable isotope data to estimate the prey contributions to the diet of each whale species, following the MixSiar model framework (Stock and Semmens 2016). We performed a separate model for each baleen whale species.

Parameters included in these models were: the $\delta^{15}N$ and $\delta^{13}C$ individual values of whales, those of their prey sources (Table S2), and the predictable shift between whale skin and diet (trophic discrimination factors) that had been previously estimated for fin whales as 2.82 ± 0.30‰ for $\delta^{15}N$ and 1.28 ± 0.38‰ for $\delta^{13}C$ (Borrell et al., 2012). The potential prey considered in the model for each whale species were identified according to previously available information on stomach content analyses conducted on whales from the North Atlantic (Table S3). Because for fin and minke whales there was detailed information on diet composition off Iceland (Víkingsson 1997; Víkingsson et al., 2014), for these two species we incorporated priors into the model. For the fin whale these were: krill: 80%, capelin: 15%, sand eel: 2.5%, and copepods: 2.5%. For the minke whale, krill: 10%, sand eel: 45%, capelin: 12.5%, herring: 12.5%, and gadoids: 20%. All models were run with the following Markov Chain Monte Carlo (MCMC) settings: length chain: 300,000–3,000,000, burn-in: 200,000–1,500,000, thin: 100, chains: 3. To ensure that all models converged we used the Gelman-Rubin and Geweke tests (see Table S4).

The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values were used to run the probabilistic method that allows defining niche region and pairwise niche overlap with three dimensions (Swanson et al., 2015) using the R package "nicheRover" (Lysy et al., 2015). For conducting the analyses, the species niche region was defined as the region with a 95% probability of finding a specific individual of that particular species and denoted as NR_{95}. For each species of baleen whale and every pair of isotopes, posterior distributions were obtained. The size of the niche and the niche overlap, defined as the probability that an individual from a particular species is found in the niche of another species (Swanson et al., 2015), were calculated. Posterior means of niche overlap and 95% credible intervals were obtained. For plotting the 5 random elliptical projections for each of the posterior distributions, the alpha value was set as 0.4 (denoted as NR_{40}) and 0.95 (denoted as NR_{95}). While NR_{40} depicts the niche core similarly to the widely used bivariate Standard Ellipse Areas (Jackson et al., 2017), NR_{95} provides the 95% probability region of the tridimensional stable isotope niche and is thus considered a more accurate measure of actual overlap. We performed 10,000 runs for all analyses.

3. Results

Table 1 shows summary statistics of the stable isotope ratios of the baleen whale species analyzed in this study. Kruskal-Wallis test showed that $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ values were significantly different among species (p.value < 0.001 for all of them). The Post-hoc Dunn test indicated that all species showed significant differences for at least one of the three stable isotope ratios, except between the blue and fin whales which did not present significant differences for any of the isotope ratios.

Table S2 details the stable isotope values of potential prey of baleen whales sampled in summer. *Calanus finmarchicus* was considered to be representative species of the copepod group and *Meganyctiphanes norvegica* of krill because these two species are the major components of their respective zooplankton groups in the region and constitute a main prey for whales (Planque and Fromentin, 1996; Víkingsson, 1997; Prieto et al., 2012). Fig. 1 shows the stable isotope ratios of both potential prey and baleen whales. Results of Bayesian mixing models indicated that krill represents the major contribution to the diet of blue (mean ± SD: 95% ± 4), fin (94% ± 7), humpback (67% ± 7) and sei (66% ± 5) whales, while it has a lower contribution in the diet of common minke whales (23% ± 10). Sand eel was the main prey for common minke whales (54% ± 15), and contributed marginally to the diet of humpback whales (7% ± 6). *C. finmarchicus* was an important prey for sei whales (34% ± 5) and had a marginal contribution in the diet of blue (5% ± 4) and fin whales (1% ± 0,4). Capelin contributed to 15% (±9) of the diet of humpback whales and to less than 10% to that of common minke

Table 1
Number of samples analyzed, mean and standard deviation of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values and niche size for each species. Values obtained from samples collected before 2013 were corrected for the Suess effect (see text). Within a column, superscript letters indicate that, according to the post-hoc Dunn test, differences between species noted with the same letter are non-significant ($p > 0.05$); e.g. $\delta^{15}N$ values showed non-significant differences between sei and fin whales (both noted with "a"), between blue and fin whales (both noted with "b"), between blue and humpback whales (both noted with "c"), and between humpback and common minke whales (both noted with "d").

Species	n	$\delta^{15}N$ (‰) mean ± SD	$\delta^{13}C$ (‰) mean ± SD	$\delta^{34}S$ (‰) mean ± SD	Niche size ($‰^3$) (α = 0.95)
Sei whale	19	8.9 ± 0.6 ^{a}	−18.7 ± 0.5 ^{a}	18.8 ± 0.3 ^{a}	4.7 ± 1.3
Blue whale	9	10.2 ± 0.4 ^{bc}	−19.5 ± 0.5^{b}	19.2 ± 0.3 ^{a}	3.7 ± 1.6
Fin whale	19	9.8 ± 0.5 ^{ab}	−19.6 ± 0.2^{b}	18.8 ± 0.4 ^{a}	3.4 ± 1.0
Humpback whale	15	11.5 ± 0.8 ^{cd}	−19.4 ± 0.7^{b}	18.3 ± 0.5^{b}	15.6 ± 5.1
Common minke whale	19	12.4 ± 1.3 ^{d}	−17.8 ± 0.5^{c}	18.3 ± 0.4^{b}	12.9 ± 3.7

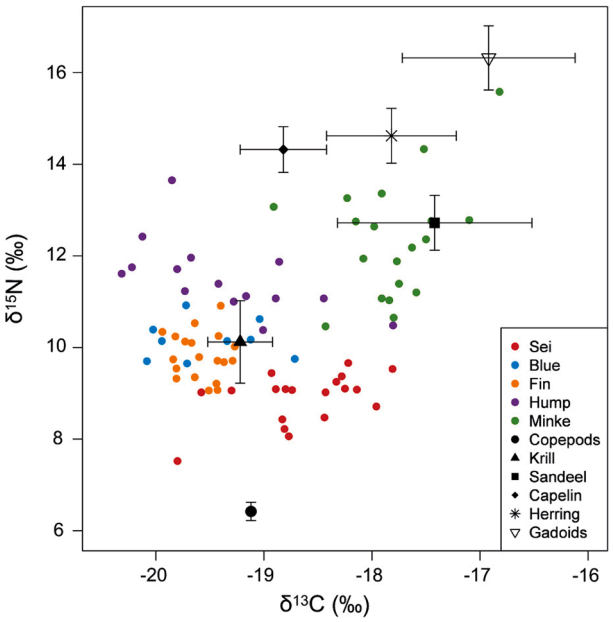


Fig. 1. Individual stable isotope ratios of N and C in the skin of baleen whales analyzed in this study, and mean (±SD) of the six potential groups of prey included in the MixSiar models corrected with the discrimination factors calculated by Borrell et al. (2012).

whales (6% ± 7). Finally, gadoids and herring accounted for less than 10% of the contribution to the diet of all the species (Fig. 2). In total, fish species contribution was the highest for common minke whales (77%), followed by humpback (34%) and fin whales (2%).

Fin whales had the smallest niche size, although it was very close to that of blue and sei whales, while common minke and humpback whales exhibited larger niche sizes (Table 1 and Fig. 3 and S1). Overlap in isotopic niches between baleen whale species was high for $\delta^{34}S$, medium for $\delta^{13}C$, and small for $\delta^{15}N$, but the result of combing the three values resulted in most cases in a moderate overlap between them. Thus, in all cases the estimated NR_{95} overlap between whale species was below 25% except between fin and blue whales, in which overlap values were between 50 and 60% (Table 2, Fig. S1).

4. Discussion

4.1. Diet composition

The stable isotope ratios determined in both the skin of the whales and in their prey are discrete measures taken from a complex scenario influenced by different variables and processes. The samples of both the whales and their prey were collected in different years, and tissue turnover, migration, the erratic movement of the whales, temporal and the geographical variation of local oceanographic conditions all interact to determine the stable isotope signal that is eventually found in organisms (e.g. Hobson and Wassenaar, 2019). As such, stable isotope ratios should be taken only as a proxy of diet and trophic interactions, and considered at the light of the knowledge on the biology of species.

Bayesian mixing models showed that sei whales primarily fed on krill, as previously reported in Iceland (Sigurjónsson 1995). *C. finmarchicus* was the second most common prey, contributing to the 34% of the diet. This highlights the importance of this species for the sei whales as it appears to be the rule in most areas of the North Atlantic, where *C. finmarchicus* or other copepods are the most abundantly consumed prey (Hjort, 1933, Flinn et al., 2002, Prieto et al., 2012, Silva et al., 2019).

The mixing models also showed that the diet of both blue and fin whales was mainly composed of krill. Although the diet of blue whales summering off Iceland has not been previously studied, our results concur with those found in other areas of the North Atlantic, such as the Estuary and Gulf of St. Lawrence or Norway (Christensen et al., 1992, Sears and Perrin 2018, Guilpin et al., 2019). In the case of the fin whale, the diet predicted by the mixing models showed that the species barely consumes fish. However, the individuals sampled were all taken during the summer, and previous studies have shown that later in the year the species also feeds on capelin (MFRI unpublished observations), coincidentally with results from other geographical regions where a significant part of the diet is composed of schooling fishes like capelin, herring, mackerel, blue whiting, and secondarily, copepods (Jonsgård 1966, Kawamura 1980, Gavrilchuk et al., 2014, Aguilar and García-Vernet 2018).

According to the mixing models, humpback whales also largely consumed krill, with fish contributing about 34% of their diet. Although there is no direct data on stomach contents from this species in Iceland (Sigurjónsson and Víkingsson 1997), this balaenopterid is usually considered a generalist species (Wright et al., 2016, Clapham 2018) and has been reported to be associated with areas of high capelin density (Pike et al., 2019). It is noteworthy that the contribution of krill in the diet of Icelandic humpback whales is much higher than that estimated for other feeding areas in the northern hemisphere, such as the Gulf of St. Lawrence (Gavrilchuk et al., 2014), Newfoundland (Piatt and Methven 1992), Norway (Christensen et al., 1992), Alaska (Wright et al., 2016) or the California current (Fleming et al., 2016). Although this difference may be reflecting the large abundance of krill during the summer in Icelandic waters and ignoring the stronger reliance of humpback whales on capelin during autumn and winter, it may also be a consequence, at least partially, of the reduction in the capelin stocks that in the last decades has taken place in these waters (Vilhjálmsson 2002, Pálsson et al., 2012a).

With regards to common minke whales, the mixing model showed that krill was still a significant component of the diet (23% of the assimilated diet) but the largest component were fish, with sand eel being the major prey and a much lower contribution of capelin, herring and gadoids. However, it should be noted that in more recent years the proportion of sand eel in the diet of common minke whales appears to have decreased, with a corresponding increase in herring and haddock purportedly by the effect of an increase in sea surface and bottom temperatures caused by global warming (Víkingsson et al., 2014). Whatever the case, the trophic level exploited by the species off Iceland is clearly higher than that of blue and fin whales, and in the upper range determined in other localities, where reliance on krill appears comparatively higher (Born et al., 2003). The composite results confirm that the common minke whale feeds on a broad range of different prey (Perrin et al., 2018) and is the most piscivorous among all baleen whale species (Skaug et al., 1997, Windsland et al., 2007).

4.2. Niche partitioning and interspecific competition

Being located at the northernmost end of the propagating wave of high productivity associated to the North Atlantic spring bloom (Visser et al., 2011), the waters off Iceland are a main summering feeding area for all the baleen whale species here examined. Baleen whales are capital breeders that migrate to temperate, low-productivity waters for reproduction (Lockyer, 1984). As a consequence, a significant portion of their annual energy budget, and in particular that required to provision their offspring during lactation, depends on the lipid reserves accrued during the intensive feeding conducted in the summering grounds (Lockyer, 1984). Trophic network analyses indicate that baleen whales are very sensitive to competition, a hypothesis that appears confirmed by the episodic appearance of emaciated individuals caused by food shortage (Moore et al., 2001, Ruzicka et al., 2013, Ribeiro et al., 2018). Niche partitioning and reduced interspecific competition while their stay in the Icelandic feeding grounds is therefore critical for the completion of their annual cycles and the long term stability of

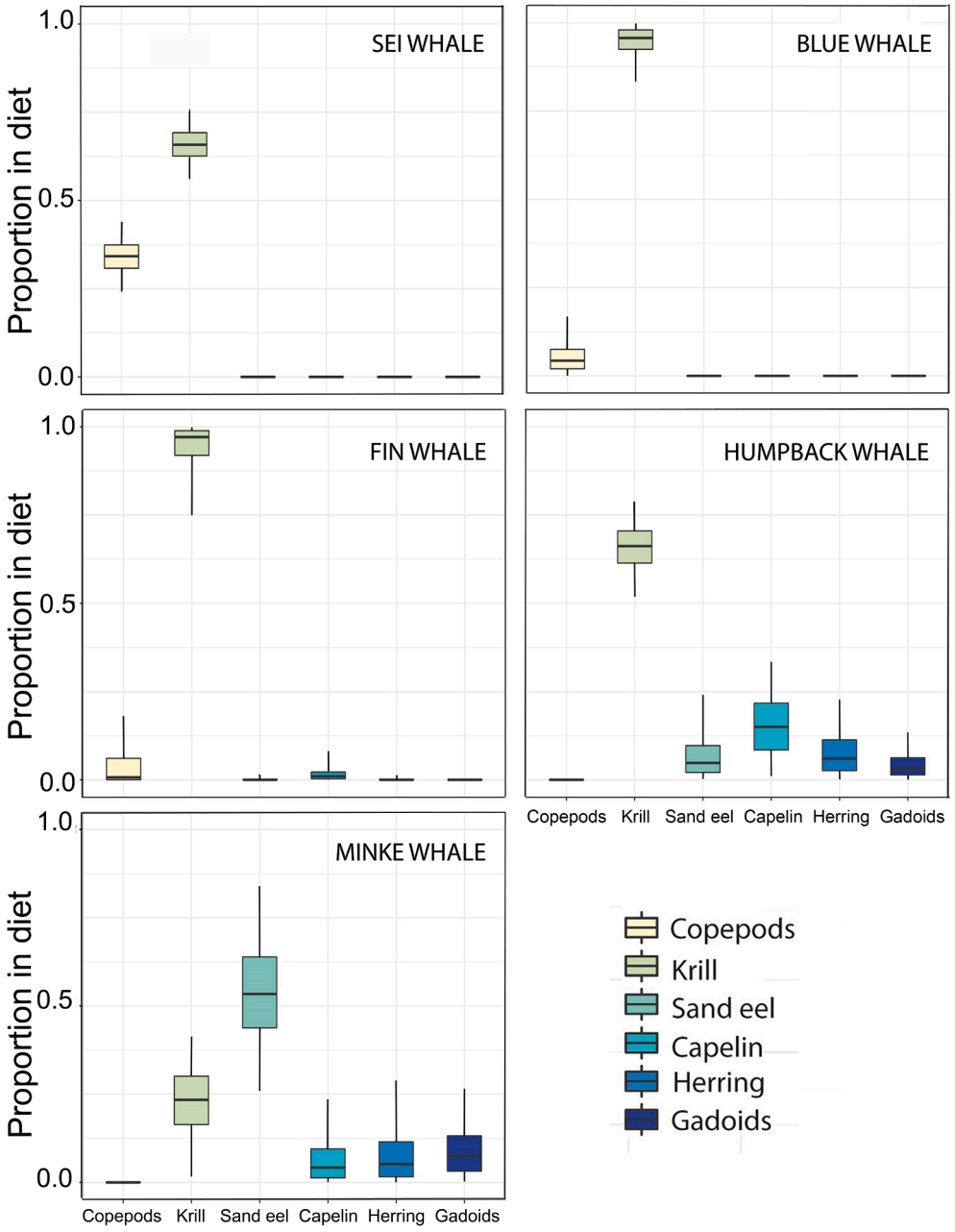


Fig. 2. Boxplot showing the estimated diet composition of the five baleen whale species studied. Proportions of the different prey are shown as 50% (inner box), 75% (outer box) and 95% credible intervals (whiskers).

populations.

The overlap in distribution, as indicated by the niches drawn by the $\delta^{13}C$ and $\delta^{34}S$ axes, is substantial and might in principle lead to a situation of diffuse competition between the various species. This would be a hindrance to coexistence because coexisting species must differ in their ecological requirements by at least some minimal amount to avoid competitive exclusion (Pianka 1974). A strong diffuse competition, as observed here, requires great average niche separation among coexisting species. This appears resolved by the niches participated by the $\delta^{15}N$ value (a trophic indicator) which separates the various species. The splitting becomes particularly clear in the niches drawn with the combination of the $\delta^{15}N$ and $\delta^{13}C$ axes, which integrate both the trophic signal with the habitat signal. The only exception to this splitting are the niches of blue and fin whales, which do not separate significantly under any combination of axes of stable isotope values.

All the species here studied consumed krill, which was always the most common prey group. This shows the strong reliance of baleen whales on this resource, as it has been previously reported in this and other areas in the North Atlantic (Jonsgård 1966, Kawamura 1980, Víkingsson 1997, Laidre et al., 2010). Indeed, the lack of distinguishability between the ecological niches of fin and blue whales is explained by their overwhelming dependence on krill. Conversely, the other species also relied substantially on other prey and their overlap in diet composition decreased to some extent.

However, diet composition is not the only factor determining ecological niche overlap. Trophic competition between sympatric species can be mitigated by segregating through other niche dimensions, either spatial and/or temporal; this is, two species may consume the same prey but forage on different size classes of the same prey (Santora et al., 2010), or forage in different locations, seasons or depths in the water column (Clapham and Brownell 1996). For example, in the Gulf of Maine both humpback whales and fin whales consume sand eel, but

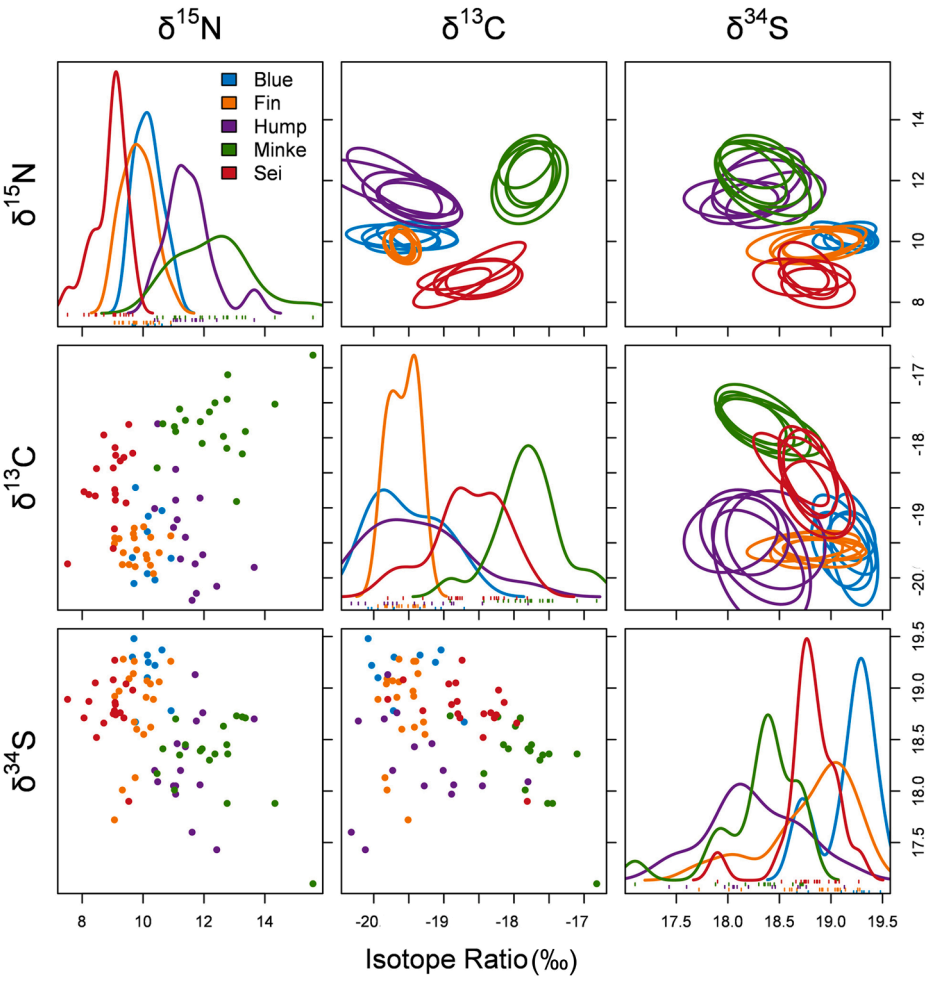


Fig. 3. NicheRover plots for the baleen whale species analyzed in this study. Top-right: five random elliptical projections at 40% niche region (NR_{40}) for each pair of isotope ratios. Diagonal: One-dimensional density plots. Bottom-left: Scatterplots of raw data for each pair of isotopes.

Table 2
Pairwise percentages of directional overlap between NR_{95} calculated using nicheROVER: posterior mean (95% credible intervals).

	Sei whale	Blue whale	Fin whale	Humpback whale	Common minke whale
Sei whale		8.1 (0.2–37.1)	5.5 (0.3–19.0)	6.1 (0–35.2)	23.5 (0.6–69.8)
Blue whale	9.2 (0.2–35.9)		57.8 (31.8–84.1)	18.9 (0.5–66.2)	10.9 (0.1–44.7)
Fin whale	8.2 (0,5–29.6)	50.2 (27.7–78.0)		14.3 (0.3–57.4)	1.2 (0–12.5)
Humpback whale	1.1 (0–5.6)	4.6 (0.1–20.1)	2.4 (0.1–9.7)		11.3 (1.8–29.4)
Common minke whale	3.9 (0.4–12.5)	2.4 (0.1–10.8)	0.2 (0–1.2)	18.1 (2.3–43.5)	

Note: The table is to be read across each row, e.g. 8.1 % of the sei whale niche overlapped the blue whale niche, and 9.2 % of the blue whale niche overlapped the sei whale niche.

hydroacoustic scans show that they exploit patches of different size or located at different depths (Clapham and Brownell 1996).

In this respect, $\delta^{34}S$ values give a clue to spatial segregation because $\delta^{34}S$ values decrease with freshwater inputs, this is, with proximity to the coast (Barros et al., 2010, Nehlich 2015). The $\delta^{34}S$ values found in blue, fin and sei whales were all relatively high and not statistically different between them, indicating that these species all forage in offshore waters. The apparent lack of difference between the blue whale and the other two balaenopterids is difficult to interpret and to a certain degree contradicts results from surveys conducted in Icelandic waters, which showed that common minke, humpback and, to less extent, blue whales are largely confined to the shelf areas, while fin and sei whales are most abundant close to the shelf slope and further out (Pike et al., 2009b, 2019; Sigurjónsson 1995). However, in other areas in the North Atlantic Ocean blue whales have been seen to forage both pelagically, over seamounts and other deep ocean structures, as well as on relatively inshore waters, such is the case of the St. Lawrence Estuary and the northwestern Gulf of St. Lawrence (Silva et al., 2013; Lesage et al., 2017). It is likely that, despite skin has a turnover rate of only few months (see above) the blue whale skin analysed still retained some of the $\delta^{34}S$ signal from previous occupancy of more offshore waters as most of the samples were taken shortly after their presumed arrival to coastal

waters. Moroever, in the last decades their distribution off Iceland has apparently experienced a northward shift that has been associated to changes in oceanographic variables and prey distribution (Víkingsson et al 2015). In such changing scenario, the matching of the evidences obtained from surveys and from stable isotope analyses may become difficult if the studies are not temporally coincidental.

Common minke whales and humpback whales presented the lowest $\delta^{34}S$ values, which indicates a more inshore distribution of these two species as compared to the rest, a finding supported by sighting studies both in Iceland and in other North Atlantic locations where all these species also co-exist (Frankel et al., 1995, Clapham 2000, Doniol-Valcroze et al., 2007, Pike et al., 2009a, 2019). Such spatial segregation again tends to reduce interspecific competition, which may be further strengthened by the differences in foraging behavior mentioned above. It is noteworthy that the niche size of common minke whales and humpback whales were the largest among all the baleen whale species here studied. This reflects the ability of these two balaenopterids to exploit a wider range of resources and habitats, something which is particularly true in the case of common minke whales, a species whose diet is well known to have pronounced spatial and temporal variation (Víkingsson et al., 2014).

A further element that strengthens resource partitioning is the timing of residence at the feeding grounds. Although the five species here examined visit Icelandic waters in the summer, their presence shows some temporal segregation. Stable isotope niches do not through light on this variable, but sightings and catch data do. Thus, Sigurjónsson and Vikingsson (1992, 1997) found that the first species arriving to the Icelandic feeding grounds are the humpback, fin and minke whales, followed by blue whales, and finally by sei whales. A similar migratory sequence has been observed off Northwestern Spain in the summer, where the peak of abundance of fin whales preceded for about 2–4 weeks the sightings of blue whales and by about 4 weeks the peak of abundance of sei whales (Aguilar and Sanpera 1982, Aguilar 1985). In other areas the same species also segregate temporarily, but the sequence may be different. At the Azores Islands in spring, the peak of abundance of blue whales preceded those of fin and sei whales (Visser et al., 2011, Silva et al., 2019), as it also happened in the North Pacific and the Antarctic, where call detections of blue whales preceded those of fin whales (Risting, 1928, Stafford et al., 2009). Taking this into account, we cannot discard that some degree of temporal segregation between the species actually occurs and slightly alleviates interspecific competition. This temporal and spatial segregation may be particularly relevant for the coexistence of blue and fin whales given the severe overlap observed in their respective stable isotope niches.

4.3. Interaction with fisheries

Some studies have suggested that marine mammals require 2–10% of the net primary production of their ecosystem, and this has triggered proposals for culling based on the alleged competition of the whales with commercial fisheries (Morissette et al., 2012, Ruzicka et al., 2013). Our results show that in Icelandic waters common minke whales and humpback whales are major predators of capelin, herring, sand eel and to a less extent of gadoids, and with little doubt, also of other species of commercial fishes that have not been included in the mixing models. This is consistent with previous estimates of food consumption by these whale species in Icelandic waters (Sigurjónsson and Víkingsson 1997) as well as from similar studies conducted in other areas of the North Atlantic (Markussen et al., 1992). Blue, fin and sei whales may also occasionally prey on fish, although their consumption rate, and therefore their direct incidence on the commercial fish stocks, appears much smaller. The abundance of each component of the trophic web is strongly interrelated and explains the observation by Víkingsson et al. (2014) that the reduction in the local stock of sand eel during the period 2003–2007 (Bogason and Lilliendahl 2008) rapidly translated into a decline in the contribution of this fish species to the diet of minke whales during the same years.

Moreover, all the baleen whale species here examined are major pelagic predators of euphausiids, and sei whales -and marginally fin and blue whales- also consume copepods, and these two groups of organisms are central elements of the macroplanktonic community in the cold waters of the Northeast Atlantic Ocean. Copepods and euphausiids feed on phytoplankton or small zooplankton and are thus at the basal levels of the trophic web which, in one way or another, sustain most commercial fish species in the region (Mauchline 1980, Astthorsson and Gislason 1997). This should be highlighted because trophic network analysis (Ruzicka et al., 2013) shows that the impact on commercial fisheries of the indirect competition for zooplankton by baleen whales is understood as being more intense than if the whales were directly preying on fish or cephalopods. Indeed, baleen whale grazing is considered to have a greater and broader potential effect on upper trophic levels and on fisheries than the specific predation by the fully piscivorous pinnipeds or odontocetes (Trites et al., 1997, Ruzicka et al., 2013). The other side of the coin is that, beyond their role and importance as macro-zooplankton or fish consumers, baleen whales also benefit fisheries by acting as food web structuring agents (Essington 2006, Willis 2007, Jusufovski et al., 2019), a fact that is valued positively for the maintenance of commercial fish stocks (Morissette et al., 2012). For example, albeit small, the segment of the baleen whale population that overwinters in Iceland (Magnusdottir and Lim 2019, Lydersen et al., 2020) may play a role of nutrient recyclers in periods of low productivity (Nicol et al., 2010, Roman and McCarthy 2010).

Interaction with fisheries involves another undesired effect, which is the potential entanglement of the whales in nets or in other fishing gear. The consequences of entanglement range from death by drowning, to stress, impaired foraging and starvation, systemic infection of unresolved entanglement wounds, and hemorrhage or debilitation due to severe gear-related damage to tissues (Cassoff et al., 2011). Even though any of the species studied here is susceptible of becoming entangled in fishing gear, the one that in other areas appears to be more strongly affected by this problem is the humpback whale due to its coastal distribution and relatively high piscivorous diet. Thus, quantification of entanglement rates using standardized scar-based techniques in the Gulf of Maine, Alaska and the Arabian Sea indicated that well over 50% of the individuals in these areas show signs of having experienced entanglements in the past (Robbins and Mattila 2004, Neilson et al., 2009, Robbins 2009). However, Basran et al. (2019) found that in Iceland the prevalence of entanglement marks in humpback whales was about half the above figures, this is, within the range 24,8–50,1%. They suggested a number of reasons to explain the difference, such as a different risk of entanglement caused by variations in the fishing gear used locally, a lower fishing pressure in the wintering or summering destinations of the whales or, because juveniles entangle more frequently than adults, geographical dissimilarities in the demographic composition. Although the question remains open, we should highlight that targeted preying on fish has been in the past associated with high entanglement rates of whales (Whitehead and Carscadden 1985). It is likely that the comparatively strong dependence on krill of the humpback whale in Iceland by may contribute, at least partially, to reduce its entanglement risk in these waters.

4.4. Evolution of interspecific relationships

A main question is how these ecological interrelationships will evolve in the future. In humpback whales, which were sampled during 2009–2013, capelin represented only 12% of the assimilated diet, a contribution that, as seen above, is much lower than what has been observed in other feeding areas of the species (Whitehead and Carscadden 1985). Without discarding the potential interaction of other factors, a possible explanation for the difference between geographical regions is the progressive warming of seawater that appears to have caused off Iceland a lower recruitment of capelin during the last decades

(Vilhjálmsson 2002, ICES 2018). To this, it should be added the northward shift in distribution of the 0-group of capelin and the westward shift of old capelin reported in the last years (Pálsson et al., 2012a), all which have resulted in a decrease of this resource off coastal Iceland during summer. In this scenario, the capelin consumers, mainly the humpback and the common minke whales, may have increased their dependence on krill and/or on other fish species not included in our analysis, a shift that has already been observed in the common minke whale of the Barents Sea (Haug et al., 2002). Such shift has been demonstrated for common minke whales in Icelandic waters with decreased krill and capelin consumption between around 1980 and after 2000, and increased proportions of herring and gadoids after the collapse of sand eel around 2005 (Víkingsson et al 2014, 2015). Also, a warming-induced mismatch in the phenology of reproduction with the peaks of oceanic productivity may have major implications on the reproductive success of some other prey species such as the sand eel, another species which is in decline in Icelandic waters (Wright et al., 2017) and that has reduced its contribution to the diet of whales (Víkingsson et al., 2014). Very likely, the changes in distribution and abundance observed in the different balaenopterid species in the region during the last decades may be a functional feeding response to the changes in the marine environment (Víkingsson et al., 2015).

The more generalist balaenopterids, particularly humpback and minke whales, have shown great plasticity to adapt in the past to varying environments (Kasamatsu and Tanaka 1992, Haug et al., 2002, Víkingsson et al., 2014, Fleming et al., 2016) and should be expected to react promptly to future changes. In recent decades there has been a significant shift in relative abundance of humpbacks whales and common minke whales in the Icelandic shelf area. While the abundance of common minke whales has drastically declined since 2001 (Pike et al., 2020), humpback whales have increased in abundance so that they have now taken over the role as the dominant baleen whale species in this area (Víkingsson et al 2015). The overlap in these two species ecological niches could indicate that inter-specific competition may have contributed to this shift. On the contrary, the more stenophagous species, like the blue whale and, to a lesser extent, the fin whale, may face difficulties if krill, their overwhelmingly basic prey, declines in abundance or varies its phenology, distribution or pattern of occurrence. Anyway, it is difficult to guess the directions that the dynamic equilibrium between the various baleen whale species will follow in a scenario of climate change. Theory predicts (Pianka 1974) that the upper limit on the permissible degree of niche overlap between species shall tend to reduce with the increasing environmental variability that it is expected to accompany global warming (Vasseur et al., 2014, Vázquez et al., 2017). This implies that, irrespective of the shifts in diet and distribution that the different species opt to, their ecological overlap will have to further reduce to allow long-term successful coexistence.

5. Conclusions

The Bayesian mixing models developed with the stable isotope data showed that, with the exception of minke whales, all baleen whales primarily fed on krill, with a variable contribution of copepods and fish depending on the species. Because baleen whales are capital breeders, the high krill availability characteristic of the feeding grounds off Iceland appears critical for these species for the completion of their migratory cycle and population maintenance. In these feeding grounds, the distribution of the various whale species substantially overlaps. This triggers strong diffuse competition which is partially mitigated by some degree of splitting in trophic niches. The only exception to this are fin and blue whales, which largely overlapped in diet and coincide in the feeding grounds during most of the feeding season. The strong dependence on krill of all species may contribute to reduce the risk of entanglement in fishing gear, particularly of humpback whales. It is unclear how the interspecific ecological relationships will evolve in the future taking into account the environmental changes observed in Icelandic waters. It has been observed some variation in the distribution of the various baleen whale species as a functional feeding response to sea water warming and salinity changes, but it is expected that the overlap in their ecological niche will have to reduce to continue allowing coexistence.

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.

Acknowledgements

The study was part of projects CGL2015-70468-R and PID2020-119712GB-I00 funded by the Ministerio de Ciencia e Innovación of Spain and the Fondo Europeo de Desarrollo Regional (FEDER). R.G-V's work was funded through a Ph.D. APIF scholarship granted by the University of Barcelona. Authors are grateful to the staff of the MFRI who conducted the fieldwork, and to Kristján Loftsson, who kindly provided facilities to conduct sampling for fin whales at the Hvalur station. The stable isotope analyses were conducted at the Centres Científics i Tecnològics (CCiT-UB) of the University of Barcelona.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pocean.2021.102690.

References

Aguilar, A., 1985. Biología y dinámica poblacional del rorcual común (*Balaenoptera physalus*) en las aguas atlánticas ibéricas. PhD thesis, University of Barcelona, 487 pp. ISBN: 84-7528-327-6.

Aguilar, A., Sanpera, C., 1982. Reanalysis of Spanish sperm, fin and sei whale catch data (1957 1980). Reports Int. Whaling Commission 32 (465), 470.

Aguilar, A., García-Vernet, R., 2018. Fin whale. In: Perrin, W.F., Würsig, B., Thewissen, J. G.M. (Eds.), Encyclopedia of Marine Mammals, third ed. pp. 368–371. https://doi.org/10.1016/B978-0-12-373553-9.00102-4.

Astthorsson, O.S., Gislason, A., 1997. Biology of euphausiids in the subarctic waters north of Iceland. Mar. Biol. 129 (2), 319–330.

Astthorsson, O.S., Valdimarsson, H., Gudmundsdottir, A., Óskarsson, G.J., 2012. Climate-related variations in the occurrence and distribution of mackerel (*Scomber scombrus*) in Icelandic waters. ICES J. Mar. Sci. 69, 1289–1297. https://doi.org/10.1093/icesjms/fss084.

Barros, N.B., Ostrom, P.H., Stricker, C.A., Wells, R.S., 2010. Stable isotopes differentiate bottlenose dolphins off west-central Florida. Mar. Mammal Sci. 26 (2), 324–336. https://doi.org/10.1111/j.1748-7692.2009.00315.x.

Basran, C.J., Bertulli, C.G., Cecchetti, A., Rasmussen, M.H., Whittaker, M., Robbins, J., 2019. First estimates of entanglement rate of humpback whales *Megaptera novaeangliae* observed in coastal Icelandic waters. Endangered Species Res. 38, 67–77. https://doi.org/10.3354/esr00936.

Best, P.B., 1993. Increase rates in severely depleted stocks of baleen whales. ICES J. Mar. Sci. 50, 169–186. https://doi.org/10.1006/jmsc.1993.1018.

Bogason, V., Lilliendahl, K., 2008. An initiation of sandeel monitoring in Iceland. Hafrannsoknir 145, 36–41.

Bogstad, B., Gjøsæter, H., Haug, T., Lindstrøm, U., 2015. A review of the battle for food in the Barents Sea: cod *vs.* marine mammals. Front. Ecol. Evolut. 3, 29. https://doi.org/10.3389/fevo.2015.00029.

Born, E.W., Outridge, P., Riget, F.F., Hobson, K.A., Dietz, R., Øien, N., Haug, T., 2003. Population substructure of North Atlantic minke whales (*Balaenoptera acutorostrata*) inferred from regional variation of elemental and stable isotopic signatures in tissues. J. Mar. Syst. 43 (1–2), 1–17. https://doi.org/10.1016/S0924-7963(03)00085-X.

Borrell, A., Abad-Oliva, N., Gómez-Campos, E., Giménez, J., Aguilar, A., 2012. Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. Rapid Commun. Mass Spectrom. 26 (14), 1596–1602. https://doi.org/10.1002/rcm.6267.

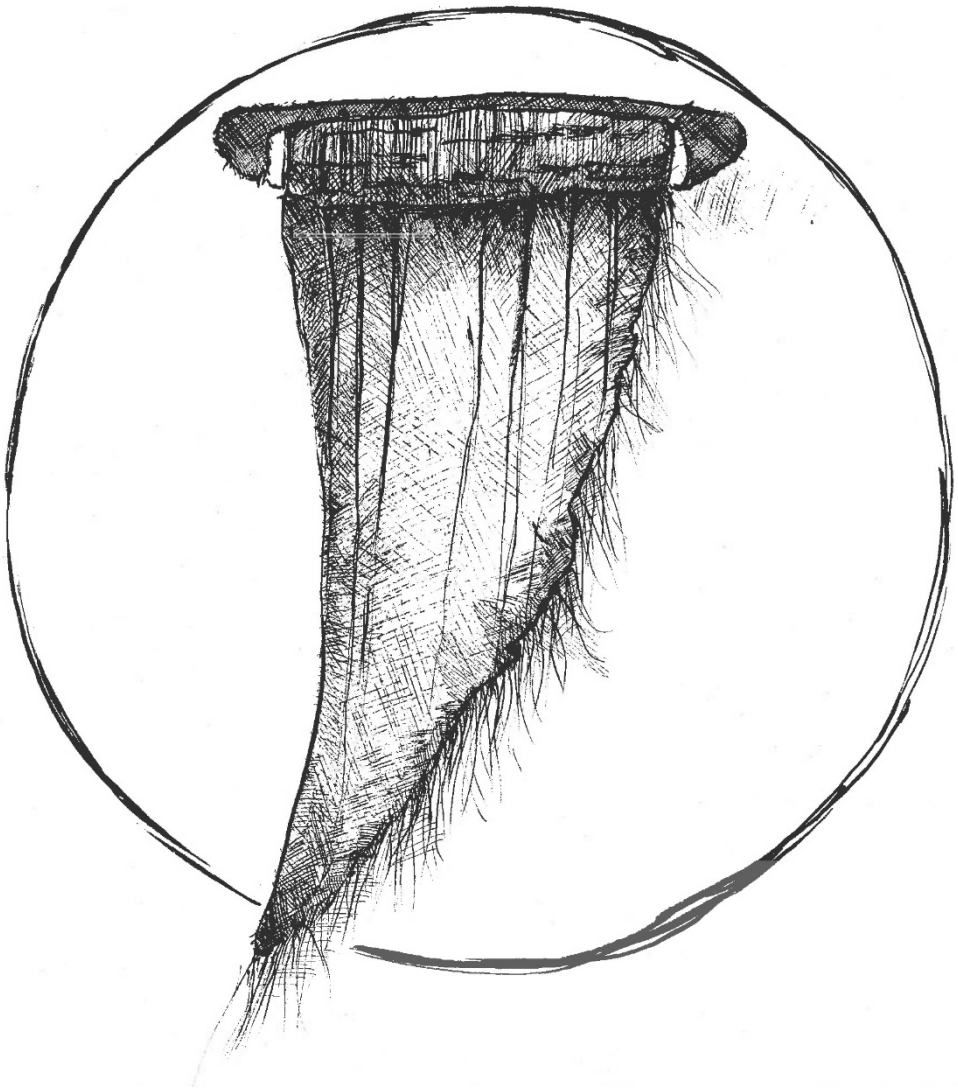
Borrell, A., Saiz, L., Víkingsson, G.A., Gaufier, P., López Fernández, A., Aguilar, A., 2018a. Fin whales as bioindicators of multi-decadal change in carbon and oxygen stable isotope shifts in the North Atlantic. Mar. Environ. Res. 138, 129–134. https://doi.org/10.1016/j.marenvres.2018.04.014.

Borrell, A., Sant, P., Víkingsson, G., Aguilar, A., García-Vernet, R., 2018b. An evaluation of whale skin differences and its suitability as a tissue for stable isotope analysis. J. Sea Res. 140, 59–62. https://doi.org/10.1016/j.seares.2018.07.011.

Borrell, A., Gazo, M., Aguilar, A., Raga, J.A., Degollada, E., Gozalbes, P., García-Vernet, R., 2021. Niche partitioning amongst northwestern Mediterranean cetaceans

using stable isotopes. Prog. Oceanogr. 193, 102559 https://doi.org/10.1016/j.pocean.2021.102559.
Bowen, W.D., Iverson, S.J., 2013. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Mar. Mammal Sci. 29 (4), 719–754. https://doi.org/10.1111/j.1748- 7692.2012.00604.x.
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 (5), 1–25. https://doi.org/10.1371/journal.pone.0177880.
Cassoff, R.M., Moore, K.M., McLellan, W.A., Barco, S.G., Rotstein, D.S., Moore, M.J., 2011. Lethal entanglement in baleen whales. Dis. Aquat. Organ. 96 (3), 175–185. https://doi.org/10.3354/dao02385.
Christensen, I., Haug, T., Øien, N., 1992. A review of feeding and reproduction in large baleen whales (Mysticeti) and sperm whales *Physeter macrocephalus* in Norwegian and adjacent waters. Fauna Norvegica, Series A 13, 39–48.
Clapham, P.J., 2000. The humpback whale: seasonal feeling and breeding in a baleen whale. In: Mann, J., Connor, R.C., Tyack, P.L., Whitehead, H. (Eds.), Cetacean Societies: Field Studies of Dolphins and Whales. University of Chicago Press, Chicago and London, pp. 173–196.
Clapham, P.J., 2018. Humpback whale. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals, third ed. https://doi.org/10.1016/b978-0-12-804327-1.00154-0.
Clapham, P.J., Brownell, R.L., 1996. The potential for interspecific competition in baleen whales. Reports Int. Whaling Commission 46, 361–367.
Clapham, P.J., Aguilar, A., Hatch, L.T., 2008. Determining spatial and temporal scales for management: lessons from whaling. Mar. Mammal Sci. 24 (1), 183–201. https://doi.org/10.1111/j.1748-7692.2007.00175.x.
Connell, J.H., 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. Am. Nat. 122 (5), 661–696.
Connolly, R.M., Guest, M.A., Melville, A.J., Oakes, J.M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia 138 (2), 161–167. https://doi.org/10.1007/s00442-003-1415-0.
DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197 (4300), 261–263. https://doi.org/10.1016/0016-7037(78)90199-0.
Doniol-Valcroze, T., Berteaux, D., Larouche, P., Sears, R., 2007. Influence of thermal fronts on habitat selection by four rorqual whale species in the Gulf of St. Lawrence. Mar. Ecol. Prog. Ser. 335, 207–216. https://doi.org/10.3354/meps335207.
Essington, T.E., 2006. Pelagic ecosystem response to a century of commercial fishing and whaling. In: Estes, J.A., DeMaster, D.P., Doak, D.F., Williams, T.M., Brownell, R.L. (Eds.), Whales, Whaling, and Ocean Ecosystems. University of California Press, Berkeley, CA, pp. 38–49.
Fleming, A.H., Clark, C.T., Calambokidis, J., Barlow, J., 2016. Humpback whale diets respond to variance in ocean climate and ecosystem conditions in the California Current. Glob. Change Biol. 22 (3), 1214–1224. https://doi.org/10.1111/gcb.13171.
Flinn, R.D., Trites, A.W., Edward, E.J., 2002. Diets of fin, sei, and sperm whales in British Columbia: an analysis of commercial whaling records, 1963–1967. Mar. Mammal Sci. 18 (6), 663–679. https://doi.org/10.1111/j.1748-7692.2002.tb01065.x.
Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Frankel, A.S., Clark, C.W., Herman, L.M., Gabriele, C.M., 1995. Spatial distribution, habitat utilization, and social interactions of humpback whales, *Megaptera novaeangliae*, off Hawai'i, determined using acoustic and visual techniques. Can. J. Zool. 73 (6), 1134–1146. https://doi.org/10.1139/z95-135.
Friedlaender, A.S., Lawson, G.L., Halpin, P.N., 2009. Evidence of resource partitioning between humpback and minke whales around the western Antarctic Peninsula. Mar. Mammal Sci. 25 (2), 402–415. https://doi.org/10.1111/j.1748-7692.2008.00263.x.
Gavrilchuk, K., Lesage, V., Ramp, C., Sears, R., Bérubé, M., Bearhop, S., Beauplet, G., 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. https://doi.org/10.3354/meps10578.
Gíslason, A., Petursdottir, H., Astthorsson, O.S., Gudmundsson, K., Valdimarsson, H., 2009. Inter-annual variability in abundance and community structure of zooplankton south and north of Iceland in relation to environmental conditions in spring 1990–2007. J. Plankton Res. 31, 541–551. https://doi.org/10.1093/plankt/fbp007.
Gómez-Campos, E., Borrell, A., Cardona, L., Forcada, J., Aguilar, A., 2011. Overfishing of small pelagic fishes increases trophic overlap between immature and mature striped dolphins in the Mediterranean Sea. PLoS ONE 6 (9), e24554. https://doi.org/10.1371/journal.pone.0024554.
Guilpin, M., Lesage, V., McQuinn, I., Goldbogen, J.A., Potvin, J., Jeanniard-du-Dot, T., Doniol-Valcroze, T., Michaud, R., Moisan, M., Winkler, G., 2019. Foraging energetics and prey density requirements of western North Atlantic blue whales in the Estuary and Gulf of St. Lawrence, Canada. Mar. Ecol. Prog. Ser. 625, 205–223. https://doi.org/10.3354/meps13043.
Hardin, G., 1960. The competitive exclusion principle. Science 131 (3409), 1292–1297. https://doi.org/10.1126/science.131.3409.1292.
Haug, T., Lindstrøm, U., Nilssen, K.T., 2002. Variations in minke whale (*Balaenoptera acutorostrata*) diet and body condition in response to ecosystem changes in the Barents Sea. Sarsia: North Atl. Mar. Sci. 87 (6), 409–422. https://doi.org/10.1080/00364820210001557l5.
Hjort, J., 1933. Whales and whaling. Hvalrådets Skrifter 7, 7–29.
Hobson, K.A., Wassenaar, L.I. (Eds.), 2019. Tracking Animal Migration with Stable Isotopes, second ed. Academic Press, 253 pp.
Huntington, H.P., 2009. A preliminary assessment of threats to arctic marine mammals and their conservation in the coming decades. Mar. Policy 33 (1), 77–82. https://doi.org/10.1016/j.marpol.2008.04.003.
Hutchinson, G.E., 1957. Concluding remarks. Cold Spring Harb. Symp. Quant. Biol. 22, 415–427. https://doi.org/10.1101/SQB.1957.022.01.039.
ICES, 2018. Capelin (*Mallotus villosus*) in subareas 5 and 14 and Division 2.a west of 5°W (Iceland and Faroes grounds, East Greenland, Jan Mayen area). 8. https://doi.org/10.17895/ices.pub.4639.
Jackson, A., Parnell, A., Jackson, M.A., 2017. Package 'SIBER'. Stable Isotope Bayesian Ellipses in R.
Jonsgård, A., 1966. Biology of the North Atlantic fin whale *Balaenoptera physalus* (L). Taxonomy, distribution, migration and food. Hvalrådets Skrifter 49, 1–62.
Jusufovski, D., Saavedra, C., Kuparinen, A., 2019. Competition between marine mammals and fisheries in contemporary harvested marine ecosystems. Mar. Ecol. Prog. Ser. 627, 207–232. https://doi.org/10.3354/meps13068.
Kasamatsu, F., Tanaka, S., 1992. Annual changes in prey species of minke whales taken off Japan 1948–87. Nippon Suisan Gakkaishi 58 (4), 637–651.
Kawamura, A., 1980. A review of food of balaenopterid whales. Sci. Rep. Whales Res. Inst., Tokyo 32, 155–197.
Keeling, C.D., 1979. The Suess effect: 13Carbon-14Carbon interrelations. Environ. Int. 2 (4–6), 229–300. https://doi.org/10.1016/0160-4120(79)90005-9.
Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78 (1), 1–27. https://doi.org/10.1139/z99-165.
Konishi, K., Tamura, T., Zenitani, R., Bando, T., Kato, H., Walløe, L., 2008. Decline in energy storage in the Antarctic minke whale (*Balaenoptera bonaerensis*) in the Southern Ocean. Polar Biol. 31, 1509–1520. https://doi.org/10.1007/s00300-008-0491-3.
Laidre, K.L., Heide-Jørgensen, M.P., Heagerty, P., Cossio, A., Bergström, B., Simon, M., 2010. Spatial associations between large baleen whales and their prey in West Greenland. Mar. Ecol. Prog. Ser. 402, 269–284. https://doi.org/10.3354/meps08423.
Lesage, V., Gavrilchuk, K., Andrews, R.D., Sears, R., 2017. Foraging areas, migratory movements and winter destinations of blue whales from the western North Atlantic. Endangered Species Res. 34, 27–43. https://doi.org/10.3354/esr00838.
Lockyer, C.H., 1984. Review of baleen whale (Mysticeti) reproduction and implications for management. Reports Int. Whaling Commission Special Issue 6, 27–50.
Lydersen, C., Vacquié-Garcia, J., 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 (1), 1–13.
Lysy, M., Stasko, A.D., Swanson, H.K., 2015. Package 'nicheROVER'. (Niche) (R)egion and Niche (Over)lap Metrics for Multidimensional Ecological Niches. 21.
MacArthur, R., Levins, R., 1967. The limiting similarity, convergence, and divergence of coexisting species. Am. Nat. 101 (921), 377–385.
Magnusdottir, E.E., Lim, R., 2019. Subarctic singers: Humpback whale (*Megaptera novaeangliae*) song structure and progression from an Icelandic feeding ground during winter. PLoS ONE 14 (1), e0210057. https://doi.org/10.1371/journal.pone.0210057.
Markussen, N.H., Ryg, M., Lydersen, C., 1992. Food consumption of the NE Atlantic minke whale (*Balaenoptera acutorostrata*) population estimated with a simulation model. ICES J. Mar. Sci. 49 (3), 317–323. https://doi.org/10.1093/icesjms/49.3.317.
Mauchline, J., 1980. The biology of the euphausiids. Adv. Mar. Biol. 18, 373–623.
McMahon, K.W., Hamady, L.L., Thorrold, S.R., 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol. Oceanogr. 58 (2), 697–714. https://doi.org/10.4319/lo.2013.58.2.0697.
Moore, S.E., Urban, R.J., Perryman, W.L., Gulland, F., Perez-Cortes, M.H., Wade, P.R., Rojas-Bracho, L., Rowles, T., 2001. Are gray whales hitting "K'' hard? Mar. Mammal Sci. 17, 954–958. https://doi.org/10.1111/j.1748-7692.2001.tb01310.x.
Moore, S.E., Haug, T., Víkingsson, G.A., Stenson, G.B., 2019. Baleen whale ecology in arctic and subarctic seas in an era of rapid habitat alteration. Prog. Oceanogr. 176, 102118 https://doi.org/10.1016/j.pocean.2019.05.010.
Mori, M., Butterworth, D.S., 2006. A first step towards modelling the krill-predator dynamics of the Antarctic ecosystem. CCAMLAR Sci. 13, 217–277.
Morissette, L., Christensen, V., Pauly, D., 2012. Marine mammal impacts in exploited ecosystems: would large scale culling benefit fisheries? PLoS ONE 7 (9), e43966. https://doi.org/10.1371/journal.pone.0043966.
Nehlich, O., 2015. The application of sulphur isotope analyses in archaeological research: A review. Earth Sci. Rev. 142, 1–17. https://doi.org/10.1016/j.earscirev.2014.12.002.
Neilson, J.L., Straley, J.M., Gabriele, C.M., Hills, S., 2009. Non-lethal entanglement of humpback whales (*Megaptera novaeangliae*) in fishing gear in northern Southeast Alaska. J. Biogeogr. 36 (3), 452–464. https://doi.org/10.1111/j.1365-2699.2007.01820.x.
Newsome, S.D., Clementz, M.T., Koch, P.L., 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Mar. Mammal Sci. 26 (3), 509–572. https://doi.org/10.1111/j.1748-7692.2009.00354.x.
Newsome, S.D., Martinez del Rio, C., Bearhop, S., Phillips, D.L., 2007. A niche for isotope ecology. Front. Ecol. Environ. 5 (8), 429–436. https://doi.org/10.1890/060150.01.
Nicol, S., Bowie, A., Jarman, S., Lannuzel, D., Meiners, K.M., van der Merwe, P., 2010. Southern Ocean iron fertilization by baleen whales and Antarctic krill. Fish Fish. 11, 203–209. https://doi.org/10.1111/j.1467-2979.2010.00356.x.
Ogle, D.H., Wheeler, P., Dinno, A., 2020. FSA: Fisheries Stock Analysis. Retrieved from https://github.com/droglenc/FSA.
Pálsson, Ó.K., Gislason, A., Guðfinnsson, H.G., Gunnarsson, B., Olafsdottir, R.S., Petursdottir, H., Sveinbjörnsson, S., Thorisson, K., Valdimarsson, H., 2012a.

Ecosystem structure in the Iceland Sea and recent changes to the capelin (*Mallotus villosus*) population. ICES J. Mar. Sci. 69 (7), 1242–1254. https://doi.org/10.4135/9781412953924.n678.
Pálsson, J., Astthorsson, O.S., Valdimarsson, H., 2012b. Hydrographic variability in Icelandic waters during recent decades and related changes in distribution of some fish species. ICES J. Mar. Sci. 69, 816–825. https://doi.org/10.1093/icesjms/fss027.
Perrin, W.F., Mallette, S.D., Brownell, R.L., 2018. Minke whales. In: Würsig, B., Thewissen, J.G.M., Kovacs, K.M. (Eds.), Encyclopedia of Marine Mammals, third ed. https://doi.org/10.1016/b978-0-12-804327-1.00175-8.
Pianka, E.R., 1974. Niche Overlap and diffuse competition. Proc. Natl. Acad. Sci. USA 71 (5), 2141–2145.
Piatt, J.F., Methven, D.A., 1992. Threshold foraging behavior of baleen whales. Mar. Ecol. Prog. Ser. 84, 205–210. https://doi.org/10.3354/meps084205.
Pike, D.G., Paxton, C.G., Gunnlaugsson, T., Víkingsson, G.A., 2009a. Trends in the distribution and abundance of cetaceans from aerial surveys in Icelandic coastal waters, 1986–2001. NAMMCO Sci. Publ. 7, 117. https://doi.org/10.7557/3.2710.
Pike, D.G., Víkingsson, G.A., Gunnlaugsson, T., Øien, N., 2009b. A note on the distribution and abundance of blue whales (*Balaenoptera musculus*) in the Central and Northeast North Atlantic. NAMMCO Sci. Publ. 7, 19. https://doi.org/10.7557/3.2703.
Pike, Daniel, Gunnlaugsson, T., Sigurjonsson, J., Vikingsson, G., 2020. Distribution and Abundance of Cetaceans in Icelandic Waters over 30 Years of Aerial Surveys. NAMMCO Sci. Publ. 11, 1–22. https://doi.org/10.7557/3.4805.
Pike, D., Gunnlaugsson, T., Mikkelsen, B., Halldórsson, S.D., Víkingsson, G., 2019. Estimates of the abundance of cetaceans in the central North Atlantic based on the NASS Icelandic and Faroese shipboard surveys conducted in 2015. NAMMCO Sci. Publ. 11 (d) https://doi.org/10.7557/3.4941.
Pinela, A.M., Borrell, A., Cardona, L., Aguilar, A., 2010. Stable isotope analysis reveals habitat partitioning among marine mammals off the NW African coast and unique trophic niches for two globally threatened species. Mar. Ecol. Prog. Ser. 416, 295–306. https://doi.org/10.3354/meps08790.
Planque, B., Fromentin, J.M., 1996. *Calanus* and environment in the eastern North Atlantic. I. Spatial and temporal patterns of *C. finmarchicus* and *C. helgolandicus*. Mar. Ecol. Prog. Ser. 134, 101–109.
Prieto, R., Janiger, D., Silva, M.A., Waring, G.T., Gonçalves, J.M., 2012. The forgotten whale: A bibliometric analysis and literature review of the North Atlantic sei whale *Balaenoptera borealis*. Mammal Rev. 42 (3), 235–272. https://doi.org/10.1111/j.1365-2907.2011.00195.x.
Ribeiro, J.P., Elvarsson, B.Þ., Sturludóttir, E., Stefánsson, G., 2018. An overview of the marine food web in Icelandic waters using Ecopath with Ecosim. arXiv preprint arXiv:1810.00613.
Risting, S., 1928. Whales and whale foetuses: statistics of catch and measurements collected from the Norwegian Whalers' Association 1922–1925. Rapports et Procès-Verbaux des Réunions 50, 1–122.
Robbins, J., 2009. Scar-based inference into Gulf of Maine humpback whale entanglement: 2003-2006. Report EA1 33F04SE0998 to the Northeast Fisheries Science Center, National Marine Fisheries Service. Center for Coastal Studies, Provincetown, MA.
Robbins, J., Mattila, D., 2004. Estimating humpback whale (*Megaptera novaeangliae*) entanglement rates on the basis of scar evidence. Report 43 EA NF030121 to the Northeast Fisheries Science Center, National Marine Fisheries Service. Center for Coastal Studies, Provincetown, MA.
Roman, J., McCarthy, J.J., 2010. The whale pump: marine mammals enhance primary production in a coastal basin. PLoS ONE 5, 1–8. https://doi.org/10.1371/journal.pone.0013255.
Ruzicka, J.J., Steele, J.H., Ballerini, T., Gaichas, S.K., Ainley, D.G., 2013. Dividing up the pie: Whales, fish, and humans as competitors. Prog. Oceanogr. 116, 207–219. https://doi.org/10.1016/j.pocean.2013.07.009.
Santora, J.A., Reiss, C.S., Loeb, V.J., Veit, R.R., 2010. Spatial association between hotspots of baleen whales and demographic patterns of Antarctic krill *Euphausia superba* suggests size-dependent predation. Mar. Ecol. Prog. Ser. 405, 255–269. https://doi.org/10.3354/meps08513.
Sarafanov, A., Sokov, A., Demidov, A., Falina, A., 2007. Warming and salinification of intermediate and deep waters in the Irminger Sea and Iceland Basin in 1997–2006. Geophys. Res. Lett. 34 (23), 1–6. https://doi.org/10.1029/2007GL031074.
Schoener, T.W., 1983. Field experiments on interspecific competition. Am. Nat. 122 (2), 240–285.
Schweder, T., Hagen, G.S., Hatlebakk, E., 2000. Direct and indirect effects of minke whale abundance on cod and herring fisheries: A scenario experiment for the greater Barents Sea. NAMMCO Sci. Publ. 2, 120–133.
Sears, R., Perrin, W.F., 2018. Blue whale. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals, third ed., pp. 110–114. https://doi.org/10.1016/B978-0-12-373553-9.00102-4.
Sigurjónsson, J., 1995. On the life history and autecology of North Atlantic rorquals. In: Blix, A.S., Walløe, L., Ulltang, Ø. (Eds.), Whales, Seals, Fish and Man. Elsevier Science, New York, USA, pp. 425–441.
Sigurjónsson, J., Galan, A., Víkingsson, G.A., 2000. A note on stomach contents of minke whales (*Balaenoptera acutorostrata*) in Icelandic waters. NAMMCO Sci. Publ. 2, 82–90.
Sigurjónsson, J., Vikingsson, G.A., 1992. Investigations on the ecological role of cetaceans in Icelandic and adjacent waters. ICES CM 1992/N,24, 23 pp.
Sigurjónsson, J., Víkingsson, G.A., 1997. Seasonal abundance of the estimated food consumption by cetaceans in Icelandic and adjacent waters. J. Northwest Atl. Fishery Sci. 22, 271–287. https://doi.org/10.2960/J.v22.a20.
Silva, M.A., Borrell, A., Prieto, R., Gauffier, P., Bérubé, M., Palsbøl, P.J., Colaço, A., 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. Roy. Soc. Open Sci. 6 (8), 181800 https://doi.org/10.1098/rsos.181800.
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 (10), e76507. https://doi.org/10.1371/journal.pone.0076507.
Silva, T., Gislason, A., Licandro, P., Marteinsdóttir, G., Ferreira, A.S.A., Gudmundsson, K., Astthorsson, O.S., 2014. Long-term changes of euphausiids in shelf and oceanic habitats southwest, south and southeast of Iceland. J. Plankton Res. 36 (5), 1262–1278. https://doi.org/10.1093/plankt/fbu050.
Skaug, H.J., Gjøsæter, H., Haug, T., Nilssen, K.T., Lindstrøm, U., 1997. Do minke whales (*Balaenoptera acutorostrata*) exhibit particular prey preferences? J. Northwest Atl. Fishery Sci. 22, 91–104. https://doi.org/10.2960/J.v22.a8.
Stafford, K.M., Citta, J.J., Moore, S.E., Daher, M.A., George, J.E., 2009. Environmental correlates of blue and fin whale call detections in the North Pacific Ocean from 1997 to 2002. Mar. Ecol. Prog. Ser. 395, 37–53. https://doi.org/10.3354/meps08362.
Stefánsson, G., Sigurjónsson, J., Víkingsson, G.A., 1997. On dynamic interactions between some fish resources and cetaceans off Iceland based on a simulation model. J. Northwest Atl. Fishery Sci. 22, 357–370 https://doi.org/10.1.1.501.9769.
Stefansdottir, L., Solmundsson, J., Marteinsdottir, G., Kristinsson, K., Jonasson, J.P., 2010. Groundfish species diversity and assemblage structure in Icelandic waters during recent years of warming. Fish. Oceanogr. 19 (1), 42–62. https://doi.org/10.1111/j.1365-2419.2009.00527.x.
Stock, B.C., Semmens, B.X., 2016. MixSIAR GUI User Manual. Version 3.1, (March), 1–42. https://doi.org/10.5281/zenodo.47719.
Swanson, H.K., Lysy, M., Power, M., Stasko, A.D., Johnson, J.D., Reist, J.D., 2015. A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. Ecology 96 (2), 318–324. https://doi.org/10.1890/14-0235.1.
Thomas, P.O., Reeves, R.R., Brownell, R.L., 2016. Status of the world's baleen whales. Mar. Mammal Sci. 32 (2), 682–734. https://doi.org/10.1111/mms.12281.
Trites, A.W., Christensen, V., Pauly, D., 1997. Competition between fisheries and marine mammals for prey and primary production in the Pacific Ocean. J. Northwest Atl. Fishery Sci. 22, 173–187.
Trites, A.W., Spitz, J., 2018. Diet. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals, third ed., pp. 255–259. https://doi.org/10.1016/B978-0-12-373553-9.00102-4.
Tulloch, V.J.D., Plagányi, É.E., Brown, C., Richardson, A.J., Matear, R., 2019. Future recovery of baleen whales is imperiled by climate change. Glob. Change Biol. 25, 1263–1281. https://doi.org/10.1111/gcb.14573.
Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk to species than climate warming. Proc. Roy. Soc. B: Biol. Sci. 281 (1779), 20132612. https://doi.org/10.1098/rspb.2013.2612.
Vázquez, D.P., Gianoli, E., Morris, W.F., Bozinovic, F., 2017. Ecological and evolutionary impacts of changing climatic variability. Biol. Rev. 92 (1), 22–42. https://doi.org/10.1111/brv.12216.
Víkingsson, G.A., 1997. Feeding of fin whales *(Balaenoptera physalus*) off Iceland – diurnal and seasonal variation and possible rates. J. Northwest Atl. Fishery Sci. 22, 77–89. https://doi.org/10.2960/J.v22.a7.
Víkingsson, G.A., Elvarsson, B.T., Ólafsdóttir, D., Sigurjónsson, J., Chosson, V., Galan, A., 2014. Recent changes in the diet composition of common minke whales (*Balaenoptera acutorostrata*) in Icelandic waters. A consequence of climate change? Mar. Biol. Res. 10 (2), 138–152. https://doi.org/10.1080/17451000.2013.793812.
Víkingsson, G.A., Pike, D.G., Valdimarsson, H., Schleimer, A., Gunnlaugsson, T., Silva, T., Elvarsson, B.Þ., Mikkelsen, B., Øien, N., Desportes, G., Bogason, V., Hammond, P.S., 2015. Distribution, abundance, and feeding ecology of baleen whales in Icelandic waters: have recent environmental changes had an effect? Front. Ecol. Evolut. 3, 1–18. https://doi.org/10.3389/fevo.2015.00006.
Vilhjálmsson, H., 2002. Capelin biology and ecology: Capelin (*Mallotus villosus*) in the Iceland-East Greenland-Jan Mayen ecosystem. ICES J. Mar. Sci. 59 (5), 870–883. https://doi.org/10.1006/jmsc.2002.1233.
Visser, F., Hartman, K.L., Pierce, G.J., Valavanis, V.D., Huisman, J., 2011. Timing of migratory baleen whales at the Azores in relation to the North Atlantic spring bloom. Mar. Ecol. Prog. Ser. 440, 267–279. https://doi.org/10.3354/meps09349.
Whitehead, H., Carscadden, J.E., 1985. Predicting inshore whale abundance— whales and capelin off the Newfoundland coast. Can. J. Fish. Aquat. Sci. 42 (5), 976–981. https://doi.org/10.1139/f85-122.
Willis, J., 2007. Could whales have maintained a high abundance of krill? Evol. Ecol. Res. 9, 651–662.
Windsland, K., Lindstrøm, U., Nilssen, K.T., Haug, T., 2007. Relative abundance and size composition of prey in the common minke whale diet in selected areas of the northeastern Atlantic during 2000–04. J. Cetacean Res. Manage. 9 (3), 167–178.
Wright, D.L., Witteveen, B., Wynne, K., Horstmann-Dehn, L., 2016. Fine-scale spatial differences in humpback whale diet composition near Kodiak, Alaska. Mar. Mammal Sci. 32 (3), 1099–1114. https://doi.org/10.1111/mms.12311.
Wright, P.J., Orpwood, J.E., Boulcott, P., 2017. Warming delays ovarian development in a capital breeder. Mar. Biol. 164 (4), 80. https://doi.org/10.1007/s00227-017-3116-y.



CAPÍTULO 2

Are stable isotope ratios and oscillations consistent in all baleen plates along the filtering apparatus? Validation of an increasingly used methodology

Autores: **Raquel García-Vernet**[a], Pol Sant[a], Gísli Víkingsson[b], Asunción Borrell[a], Alex Aguilar[a]

[a] *Departamento de Biología Evolutiva, Ecología i Ciencias Ambientales, y IRBio, Facultad de Biología, Universidad de Barcelona*

[b] *Marine and Freshwater Research Institute, Fornubúðum 5, 220 Hafnarfjörður, Iceland*

Rationale: Baleen plates are anatomical structures composed of inert tissue that hang from the upper jaw in mysticetes. Baleen plates may differ in size and in coloration between different segments of the filtering row or between sides of the mouth. Concern has been raised that variation in baleen plate characteristics may reflect dissimilar structural composition and growth rates liable to affect stable isotope ratios and their oscillation patterns.

Methods: We measured stable carbon (δ13C values) and nitrogen (δ15N values) isotope ratios at intervals of 1 cm along the longitudinal axis of six baleen plates collected from different positions along the mouth of a fin whale. All samples were analysed using a continuous flow isotope ratio mass spectrometer. Generalized additive models were fitted to the data from each baleen plate and the results of the models were compared visually.

Results: A total of 206 samples were analysed. Visually, all baleen plates presented nearly identical oscillations, independent of the position or the coloration of the baleen plate. However, the variation in δ13C and δ15N values occurring between the different baleen plates was higher in the segments of oscillations exhibiting steeper slopes.

Conclusions: Differences in size between plates in an individual are due to differential erosion rates according to their position in the mouth. Therefore, the position of sampling along the baleen plate row should not be a reason for concern when conducting stable isotope studies.

Received: 27 November 2017 | Revised: 9 May 2018 | Accepted: 9 May 2018

DOI: 10.1002/rcm.8169

RESEARCH ARTICLE

WILEY Rapid Communications in Mass Spectrometry

Are stable isotope ratios and oscillations consistent in all baleen plates along the filtering apparatus? Validation of an increasingly used methodology

Raquel García-Vernet[1] | Pol Sant[1] | Gísli Víkingsson[2] | Asunción Borrell[1] | Alex Aguilar[1]

[1] Institute of Biodiversity Research (IRBio) and Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain

[2] Marine and Freshwater Research Institute, PO Box 1390, Skúlagata 4, 121, Reykjavík, Iceland

Correspondence
Raquel García-Vernet, Institute of Biodiversity Research (IRBio) and Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain.
Email: rgarciaver@ub.edu

Funding information
Ministerio de Economía y Competitividad, Grant/Award Number: CGL2015-70468-R

Rationale: Baleen plates are anatomical structures composed of inert tissue that hang from the upper jaw in mysticetes. Baleen plates may differ in size and in coloration between different segments of the filtering row or between sides of the mouth. Concern has been raised that variation in baleen plate characteristics may reflect dissimilar structural composition and growth rates liable to affect stable isotope ratios and their oscillation patterns.

Methods: We measured stable carbon ($\delta^{13}C$ values) and nitrogen ($\delta^{15}N$ values) isotope ratios at intervals of 1 cm along the longitudinal axis of six baleen plates collected from different positions along the mouth of a fin whale. All samples were analysed using a continuous flow isotope ratio mass spectrometer. Generalized additive models were fitted to the data from each baleen plate and the results of the models were compared visually.

Results: A total of 206 samples were analysed. Visually, all baleen plates presented nearly identical oscillations, independent of the position or the coloration of the baleen plate. However, the variation in $\delta^{13}C$ and $\delta^{15}N$ values occurring between the different baleen plates was higher in the segments of oscillations exhibiting steeper slopes.

Conclusions: Differences in size between plates in an individual are due to differential erosion rates according to their position in the mouth. Therefore, the position of sampling along the baleen plate row should not be a reason for concern when conducting stable isotope studies.

1 | INTRODUCTION

In the few last decades, stable isotope analysis has become a standard tool in animal ecology studies, particularly to investigate diet composition, migration and physiology of individuals in the wild.[1,2] In marine mammals, this technique has experienced substantial development[3] because these animals are difficult to observe or handle, and therefore many of their biological traits can only be determined through the application of chemical markers. The stable isotope ratios of nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$ values) and carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$ values) are markers commonly used because they inform about diet, trophic level and characteristics of the ecosystem in which an animal feeds.[4-7]

Stable isotope studies can be carried out on any body tissue, although each tissue has different discrimination factors and turnover rates.[8-10] Some bones, otoliths and teeth, as well as keratinous tissues such as feathers, hair, nails or baleen plates, are biologically inert, which means that their biogeochemical composition does not vary after the tissue is consolidated. In the cases in which such tissues experience continuous growth, a chronologically sequential record of the environment in which the animal has lived is preserved in successive growth layers. This property has been used to infer variations in physiology or habitat use during periods of the life cycle of individuals that otherwise would be impossible to monitor.[11-15]

Baleen plates grow continuously and therefore they sequentially archive the stable isotopic seascape of the water mass in which a whale lives or its variation in feeding regimes.[16-18] Schell et al were the first to measure stable isotopes ratios along the growth axis of a baleen plate, obtaining a temporal record of recent movements and diet.[19] Since then, many studies have followed this approach to gain information on migration and diet shifts in a variety of baleen whale species.[17,20-23] Because many whale species or populations stay during part of their life cycle in unknown geographical destinations, baleen plates provide an invaluable insight into these periods that otherwise would remain obscure.

Baleen plates hang from the upper jaw in bilateral rows along the rostrum and, depending on their position, they greatly vary in size; those in the centro-posterior region are the largest, with sizes diminishing caudally and distally.[24] In addition, the color of the plates varies between species but, more importantly, in some baleen whales the color of the baleen plates may vary between different segments of the row or between sides of the mouth. The heterogeneity in size according to position in the mouth may be due to differences in the baleen plate growth rates. If this were the case, the amplitude of the oscillations of the stable isotope ratios along a baleen plate would differ between plates of different size. In addition, differences in coloration may reflect dissimilar structural composition, also potentially affecting the isotope ratios. Concern about these issues has been expressed in some previous studies but they have never been addressed through specifically designed experiments, thus remaining unresolved.[19,25-28]

With the aim of optimizing the use of baleen plates to investigate the ecology of mysticetes, we investigate here the potential effect that non-standardized sampling of baleen plates may have on stable isotope ratios and their oscillations along the plates. We have examined the replicability of stable isotope patterns between the baleen plates of the same fin whale, but occupying different positions in the mouth and thus having dissimilar size and coloration. The fin whale was selected because, as with most baleen whales, it undertakes annual migrations alternating high-latitude summer grounds with low-latitude winter grounds[29] and clear oscillations of the stable isotope ratios have been observed in their baleen plates.[17,27,30] In addition, the fin whale is the mysticete in which the coloration of baleen plates shows the highest heterogeneity and asymmetry,[29] thus permitting us to test for the potential effect of bilaterality or coloration-related differences.

2 | EXPERIMENTAL

2.1 | Sample collection and preparation

The baleen plates were obtained from a 17.40 m male fin whale flensed at the Hvalur H/F whaling station (Hvalfjordur, Iceland) on 8 August 2015. The samples were transported internationally under CITES permit numbers 15IS017MA and ESBB00207/15I. The length of the baleen filtering apparatus on the right-hand side of the mouth was measured and five plates were collected in roughly equidistant positions from the tip – identified as A, B, C, D and E (Figure 1). An additional plate, identified as O, was collected from the left maxilla in the position equivalent to position C.

The baleen plates were labelled and initially preserved at −20°C. Once at the laboratory, they were thawed, the gum was removed with steel wool to allow adequate sampling of the keratin plate, and the surface of the plate was cleaned for external or adhered materials using steel wool and a chloroform–methanol solution (2:1). Once clean, the plates were stored dry until analysis. The subsamples used for the stable isotope analysis were extracted with a grinder delineating parallel rows separated by 1 cm and starting from the proximal part of the baleen (that most recently formed) to the most distal (the oldest part of the plate). The number of subsamples varied between plates according to their length.

2.2 | Stable isotope analysis

Approximately 0.3 mg of powered subsamples was weighed into tin capsules. Samples were automatically loaded and combusted at 1000°C to be analyzed using a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Flash 1112 elemental analyzer; CE Elantech, Lakewood, NJ, USA), coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (both from ThermoFinnigan, Bremen, Germany). International isotope secondary standards of known $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, namely polyethylene (IAEA CH7; $\delta^{13}C$ = − 31.8‰), sucrose (IAEA CH6; $\delta^{13}C$ = −10.4‰), ammonium sulfate (IAEA N1; $\delta^{15}N$ = +0.4‰ and IAEA N2; $\delta^{15}N$ = +20.3‰), potassium nitrate (USGS 34; $\delta^{15}N$ = −1.7‰), L-glutamic acid (USGS 40; $\delta^{15}N$ = −4.6‰; $\delta^{13}C$ = −26.2‰) and caffeine (IAEA 600; $\delta^{15}N$ = 1.0‰; $\delta^{13}C$ = −27.7‰), were used to calibrate the system and compensate for any analytical drift over time. The reference materials used for the analysis were selected according to previous calibration experiments performed on the same type of samples to ensure that the range of the reference values spanned those of the samples.

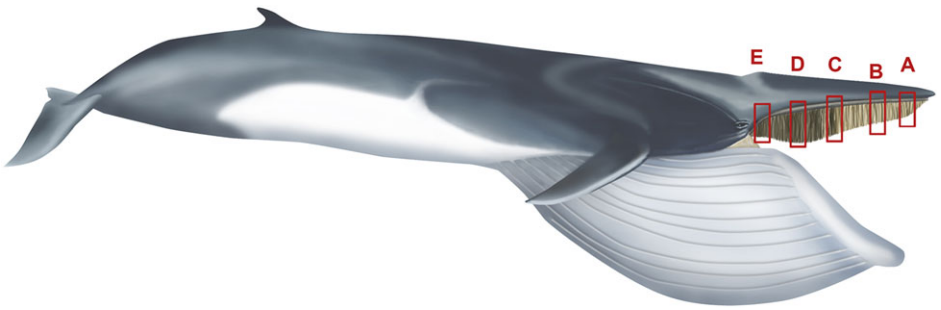


FIGURE 1 Place of sampling of each plate. The total length of the filtering apparatus was 362 cm. The five plates from the right maxilla were collected from roughly equidistant positions from the tip: A at 45 cm, B at 90 cm, C at 180 cm, D at 270 cm and E at 316 cm. Plate O was collected from the left maxilla at the position equivalent to position C [Color figure can be viewed at wileyonlinelibrary.com]

Stable isotopes ratios are expressed following the delta (δ) notation, while the relative variations of stable isotope ratios are expressed as per mil (‰) deviations from the predefined international standards according to the equation:

$$\delta X = \frac{R_{sample}}{R_{standard}} - 1$$

where X is ^{13}C or ^{15}N, and R_{sample} and $R_{standard}$ are the heavy-to-light isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and in the reference standards, respectively. These standards are the Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N. The precision and accuracy for $\delta^{13}C$ and $\delta^{15}N$ measurements were 0.1 and 0.3‰, respectively. These analyses were conducted at the Centres Cientifics i Tecnològics of the University of Barcelona (CCiT-UB).

2.3 | Data analysis

With the aim of visually comparing oscillations between the baleen plates, the isotopic ratios of carbon and nitrogen were individually examined by fitting a generalized additive model (GAM) to the data from each baleen plate using the mgcv package[31] in R.[32] Each model was fitted by considering the isotope ratios of each element as the dependent variable and the length of the different baleen plates as the independent variable. For each baleen plate and isotope ratio, homoscedasticity and normality of the residuals were checked, and models were adjusted by removing outliers and choosing the best *k*. All the parameters are specified in Table S1 (supporting information). Finally, results of the models were plotted to visually compare the oscillations in each baleen plate.

The $\delta^{15}N$ and $\delta^{13}C$ values were examined in the first 18 cm (starting from the gum) of each plate, which roughly included the most recent complete migratory cycle of the whale.[17] Data were tested for normality (Shapiro–Wilk test) and homoscedasticity (Levene's test), and means and standard deviations were calculated for each baleen plate. To investigate whether the variability between plates was constant along the whole plate length, standard deviation values for each data point were calculated and plotted (Figure 2).

3 | RESULTS

A total of 206 samples were analyzed. The pigmentation and number of points analyzed for each baleen plate, as well as the mean and standard deviations of $\delta^{15}N$ and $\delta^{13}C$ values in the first 18 cm of each baleen plate, are detailed in Table 1. Standard deviations for each data point along the first 18 cm of baleen plate length are shown in Figure 2. Almost all data points had standard deviations around or below 0.3, in agreement with the analytical precision of the $\delta^{13}C$ and $\delta^{15}N$ measurements (see section 2). Despite this general trend, some segments of the baleen plates showed higher standard deviations. For $\delta^{15}N$ values this occurred in the first 3 data points situated in the proximal part of the baleen plate, and for $\delta^{13}C$ values this occurred in the data points 14 and 15. In both cases these points coincide with the segments of the baleen plates where the $\delta^{15}N$ and

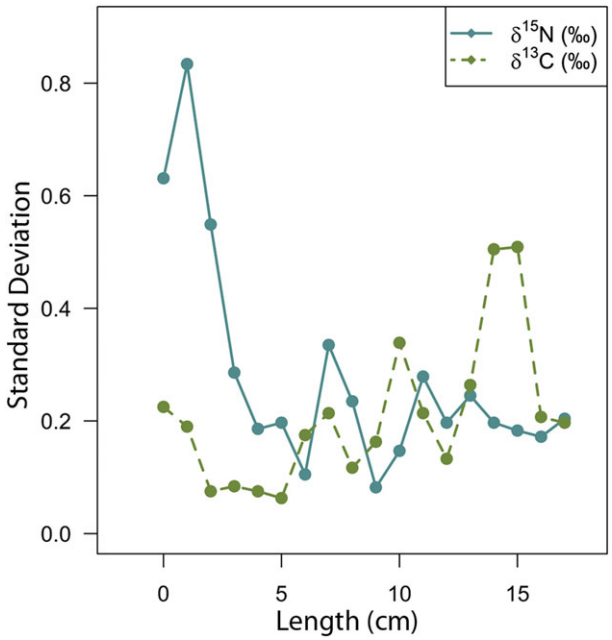


FIGURE 2 Standard deviation for $\delta^{13}C$ (green) and $\delta^{15}N$ values (blue) for each sampling point along the growing axis of the baleen plates [Color figure can be viewed at wileyonlinelibrary.com]

$\delta^{13}C$ values undergo a rapid change (Figure 3). All the baleen plates showed oscillations in their $\delta^{13}C$ and $\delta^{15}N$ values along their growing axis (Figure 3), and the trends were nearly identical in all baleen plates.

4 | DISCUSSION

Validation and standardization of the sampling of archival tissues to infer ecological and physiological traits, variation in diet or migration have been conducted in a number of species and for a variety of keratinous structures, such as human and other animal hair, pinniped vibrissae or bird feathers.[11,33-35] These studies have involved assessment of variability within individuals and within repeated samples of the same individual. However, possibly because of the difficulty of acquiring adequate samples, baleen plates have not so far been subject to extensive methodological studies despite expressed concerns about the potential non-replicability between baleen plates from the same individual.

Schell et al[19] and Lubetkin et al[26] investigated oscillations between two opposite plates in a bowhead whale (*Balaena mysticetus*), and Caraveo-Patiño and Soto[25] compared two consecutive plates in a

TABLE 1 Characteristics of plates analysed in this study and $\delta^{15}N$ and $\delta^{13}C$ values (mean ± standard deviation) for the first 18 cm

Plate	Pigmentation	No. samples	$\delta^{15}N$ ± s.d. (‰)	$\delta^{13}C$ ± s.d. (‰)
A	Yellowish	20	9.7 ± 0.7	−18.4 ± 0.5
B	Yellowish	29	9.8 ± 0.5	−18.7 ± 0.5
C	Grey	45	9.8 ± 0.5	−18.8 ± 0.5
D	Grey	34	9.8 ± 0.4	−18.9 ± 0.5
E	Slate grey	34	10.1 ± 0.4	−18.7 ± 0.5
O	Grey	44	9.9 ± 0.4	−18.7 ± 0.5

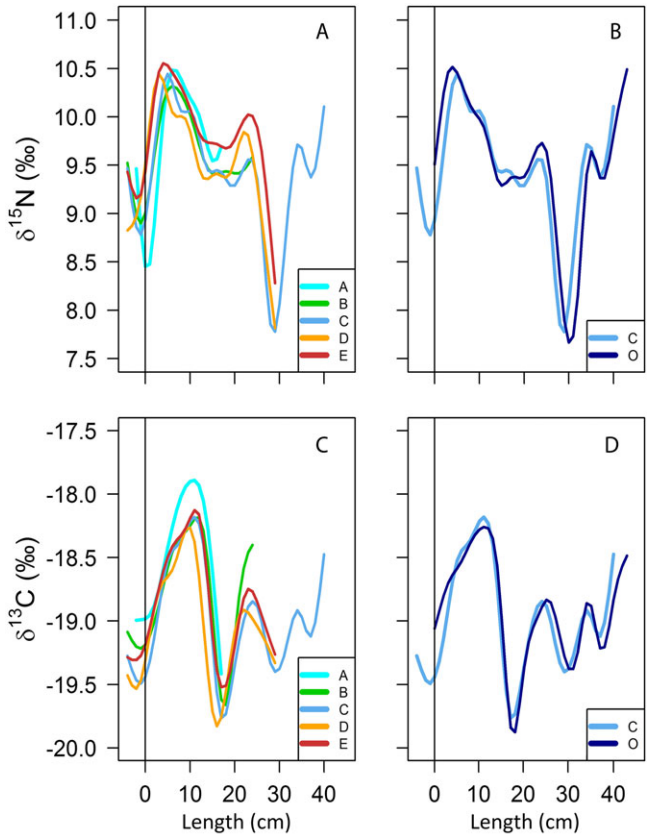


FIGURE 3 Results of the GAM fitted for each baleen plate for $\delta^{15}N$ values (A, B) and $\delta^{13}C$ values (C, D) along the growing axis of the various plates from the left row (A, C), and comparison of plates occupying central positions in each body side: C the left-hand side and O in the right-hand side (B, D) [Color figure can be viewed at wileyonlinelibrary.com]

grey whale (*Eschrichtius robustus*). In all cases the oscillations found in the various plates were very similar, although the plates selected had in all cases been obtained from approximately the centro-posterior part of the filtering apparatus, where the size of the plates is larger. As a consequence, the potential effect of differential growth rates according to plate size or position in the maxilla, if occurring, could not be appropriately tested. Only two studies, that of Eisenmann et al[28] in humpback whales (*Megaptera novaeangliae*) and that of Bentaleb et al[27] in fin whales, have compared pairs of baleen plates of different size belonging to the same individual, obtaining in the two cases dissimilar results: the first found nearly identical patterns in each pair of plates, while the second found different oscillations between the plates although the mean values of $\delta^{15}N$ and $\delta^{13}C$ for corresponding segments of the plates were found to be similar.

With the aim of clarifying this issue, we analyzed six baleen plates collected from different positions in the mouth of the same animal. The highest variability between plates was found in segments of the baleen plates in which the change in the stable isotope ratios occurs fast (Figures 2 and 3). Due to such rapid modification in the stable isotope ratios, small variations in determining the sampling location probably produce large differences in the stable isotope ratio results. To overcome this, we suggest that the segments of the baleen plates subject to faster changes in stable isotope ratios should be sampled at smaller intervals (e.g. a few millimetres apart) than the rest of the segments.

Nonetheless, the stable isotope ratios observed throughout the baleen plates of different sizes, and sampled in different positions along the filtering apparatus, presented nearly identical oscillations (Figure 3). This similarity among isotopic patterns indicates that all baleen plates grow at similar rates and that differences in plate size are due to the differential erosion to which the plates are subjected according to their position in the mouth, as has historically been assumed.[36,37] Records of variation in thickness in different baleen plates proceeding from a single animal suggest that short plates present the same pattern as the long plates' upper part. Thus, shorter baleen plates seem to be exposed to greater erosion than longer plates.[38] However, until now this hypothesis had only been confirmed in grey whales.[39,40]

Another potential source of heterogeneity in sampling between plates is coloration. A number of mysticetes show some degree of asymmetry in body pigmentation, and different segments of the filtering apparatus may show dissimilar plate coloring. In the fin whale (*Balaenoptera physalus*) the asymmetry is extremely marked: in the left-hand side, the lower jaw is dark grey and the plates are grey, while in the right-hand side the lower jaw is white and the rear two-thirds of the plates are grey but those on the front third are yellowish.[29] In the sei whale (*Balaenoptera borealis*), most baleen plates are dark grey but those in the front tend to be whitish.[41] In the dwarf minke whale, *Balaenoptera acutorostrata*,[42] and in Omura's whale, *Balaenoptera omurai*, baleen plates do not show marked asymmetry but the coloration of the head does, although in the latter species the asymmetry is reversed from that in the fin whale: the lower jaw area is black on the left side and white on the right.[43] The reasons for the differences in coloration and size of the plates are unclear, but it is generally accepted that they reflect dissimilarities in function between mouth segments or sides.[44]

The potential effect of pigmentation on the stable isotope ratios has been investigated in the skin of various species of cetaceans. Thus, the ratios in the dorsal region of the body trunk (typically dark-colored) have been compared with those in the ventral region (typically white or pale-coloured) in striped (*Stenella coeruleoalba*) and common dolphins (*Delphinus delphis*),[45] bottlenose dolphins (*Tursiops truncatus*) and killer whales (*Orcinus orca*),[46] and in all cases the resulting ratios were statistically undistinguishable. In addition, studies in human hair have shown that loss of pigment has no effect on the C/N, $\delta^{15}N$ and $\delta^{13}C$ values,[47,48] all indicating that coloration *per se* should not be expected to have any effect on stable isotope ratios.

The asymmetric coloration of both the rostral region and the baleen plates that occurs in some mysticetes is commonly thought to serve in the maintenance of the countershading when the whale rolls to its side during feeding lunges, or to aid in startling prey and elicit its aggregation.[49,50] However, this hypothesis does not appear to be clearly supported by field data.[44] If the asymmetric variation is limited to pigmentation, the above evidences from skin and hair would point to a non-effect on stable isotope ratios of baleen plates of different coloration. However, it can be reasonably argued that the evolutionary forces that have brought different segments of the baleen plate rows, or of different sides of the mouth, to acquire dissimilar colorations may also reflect differences in function of the filtering apparatus and therefore may have also affected the

mechanical properties of the baleen and its structure, rate of growth or rate of erosion. Thus, tendency to selectively roll to one side or another during feeding may induce differential mechanical tensions or differential erosion to the plates in each body side. Independent of whether this is true or not, the results of the present study show that the stable isotope ratios and their oscillation patterns were indistinguishable either between plates displaying contrasting coloration or between plates sampled in the same position of the filtering row but collected from opposite sides of the mouth.

5 | CONCLUSIONS

We can conclude that all baleen plates, independent of their position in the filtering apparatus, size or coloration, grow at the same rate and display similar stable isotope ratios and oscillations. Differences in size between plates in a same individual are thus solely due to differential erosion rates depending on the position of the baleen plates in the mouth. Therefore, position of sampling along the baleen plate row should not be a significant source of concern with regard to sampling for stable isotope studies. However, in the segments where stable isotope ratios change rapidly, it is recommended that sampling should take place at smaller intervals than in the other segments to obtain a precise trend of the isotopic ratios along the whole plate. In addition, with the aim of optimizing and standardizing procedures, it is recommended that whenever possible baleen plates should be sampled in the central position of the left row, which in most species is dark-colored and among the largest in the filtering apparatus, thus providing the longest time span for investigating seasonal oscillations.

ACKNOWLEDGEMENTS

Samples were provided by the Marine Research Institute of Iceland (Hafrannsóknastofnun). The authors are grateful to the personnel of the Marine Research Institute who conducted the fieldwork, as well as to the staff of the Centres Científics i Tecnològics (CCiT-UB) of the University of Barcelona, where the stable isotope ratio determinations were carried out. This work was supported by project CGL2015-70468-R (MINECO/FEDER, UE).

ORCID

Raquel García-Vernet http://orcid.org/0000-0001-6717-1654
Asunción Borrell http://orcid.org/0000-0002-6714-0724
Alex Aguilar http://orcid.org/0000-0002-5751-2512

REFERENCES

1. Hobson KA. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*. 1999;120:314-326. https://doi.org/10.1007/s004420050865
2. Kelly JF. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can J Zool*. 2000;78:1-27. https://doi.org/10.1139/z99-165
3. Newsome SD, Clementz MT, Koch PL. Using stable isotope biogeochemistry to study marine mammal ecology. *Mar Mamm Sci*. 2010;26(3):509-572. https://doi.org/10.1111/j.1748-7692.2009.00354.x
4. DeNiro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta*. 1981;45:341-351. https://doi.org/10.1016/0016-7037(81)90244-1
5. Peterson BJ, Fry B. Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst*. 1987;18:293-320. https://doi.org/10.1146/annurev.es.18.110187.001453
6. Fry B. Food web structure on Georges Bank from stable C, N and S isotopic compositions. *Limnol Oceanogr*. 1988;33:1182-1190. https://doi.org/10.4319/lo.1988.33.5.1182
7. Hobson KA, Piatt JF, Pitocchelli J. Using stable isotopes to determine seabird trophic relationships. *J Anim Ecol*. 1994;63:786-798. https://doi.org/10.2307/5256
8. Hobson KA, Schell DM, Renouf D, Noseworthy E. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can J Fish Aquat Sci*. 1996;53:528-533. https://doi.org/10.1139/cjfas-53-3-528
9. Caut S, Angulo E, Courchamp F. Variation in discrimination factors ($\delta^{15}N$ and $\delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol*. 2009;46:443-453. https://doi.org/10.1111/j.1365-2664.2009.01620.x
10. Borrell A, Abad-Oliva N, Gómez-Campos E, Giménez J, Aguilar A. Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. *Rapid Commun Mass Spectrom*. 2012;26:1596-1602. https://doi.org/10.1002/rcm.6267
11. Ramos R, González-Solís J. Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. *Front Ecol Environ*. 2012;10(5):258-266. https://doi.org/10.1890/110140
12. Rooker JR, Secor DH, De Metrio G, Schloesser R, Block BA, Neilson JD. Natal homing and connectivity in Atlantic bluefin tuna populations. *Science*. 2008;322(5902):742-744. https://doi.org/10.1126/science.1161473
13. Cherel Y, Kernaléguen L, Richard P, Guinet C. Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. *Biol Lett*. 2009;5:830-832. https://doi.org/10.1098/rsbl.2009.0552
14. Borrell A, Vacca AV, Pinela AM, et al. Stable isotopes provide insight into population structure and segregation in eastern North Atlantic sperm whales. *PLoS ONE*. 2013;8(12):e82398. https://doi.org/10.1371/journal.pone.0082398
15. Matthews CJD, Longstaffe FJ, Ferguson SH. Dentine oxygen isotopes ($\delta^{18}O$) as a proxy for odontocete distributions and movements. *Ecol Evol*. 2016;6(14):4643-4653. https://doi.org/10.1002/ece3.2238
16. Schell DM, Saupe SM. Feeding and growth as indicated by stable isotopes. In: Burns JJ, Montague JJ, Cowles CJ, eds. *The Bowhead Whale*. Lawrence, KS: Allen Press; 1993:491-509.
17. Aguilar A, Giménez J, Gómez-Campos E, Cardona L, Borrell A. $\delta^{15}N$ value does not reflect fasting in mysticetes. *PLoS ONE*. 2014;9(3):e92288. https://doi.org/10.1371/journal.pone.0092288
18. Mitani Y, Bando T, Takai N, Sakamoto W. Patterns of stable carbon and nitrogen isotopes in the baleen of common minke whale *Balaenoptera acutorostrata* from the western North Pacific. *Fish Sci*. 2006;72(1):69-76. https://doi.org/10.1111/j.1444-2906.2006.01118.x
19. Schell DM, Saupe SM, Haubenstock N. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by $\delta^{13}C$ techniques. *Mar Biol*. 1989;103:433-443. https://doi.org/10.1007/BF00399575
20. Best PB, Schell DM. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. *Mar Biol*. 1996;124:483-494. https://doi.org/10.1007/BF00351030
21. Lee SH, Schell DM, McDonald TL, Richardson WJ. Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. *Mar Ecol Prog Ser*. 2005;285:271-287. https://doi.org/10.3354/meps285271

22. Caraveo-Patiño J, Hobson KA, Soto LA. Feeding ecology of gray whales inferred from stable-carbon and nitrogen isotopic analysis of baleen plates. *Hydrobiologia*. 2007;586:17-25. https://doi.org/10.1007/s10750-006-0477-5
23. Matthews CJD, Ferguson SH. Seasonal foraging behaviour of eastern Canada–west Greenland bowhead whales: an assessment of isotopic cycles along baleen. *Mar Ecol Prog Ser*. 2015;522:269-286. https://doi.org/10.3354/meps11145
24. Fudge DS, Szewciw LJ, Schwalb AN. Morphology and development of blue whale baleen: an annotated translation of Tycho Tullberg's classic 1883 paper. *Aquat Mamm*. 2009;35:226-252. https://doi.org/10.1578/AM.35.2.2009.226
25. Caraveo-Patiño J, Soto LA. Stable carbon isotope ratios for the gray whale (*Eschrichtius robustus*) in the breeding grounds of Baja California Sur, Mexico. *Hydrobiologia*. 2005;539:99-107. https://doi.org/10.1007/s10750-004-3370-0
26. Lubetkin SC, Zeh JE, Rosa C, George JC. Age estimation for young bowhead whales (*Balaena mysticetus*) using annual baleen growth increments. *Can J Zool*. 2008;86:525-538. https://doi.org/10.1139/Z08-028
27. Bentaleb I, Martin C, Vrac M, et al. Foraging ecology of Mediterranean fin whales in a changing environment elucidated by satellite tracking and baleen plate stable isotopes. *Mar Ecol Prog Ser*. 2011;438:285-302. https://doi.org/10.3354/meps09269
28. Eisenmann P, Fry B, Holyoake C, Coughran D, Nicol S, Nash SB. Isotopic evidence of a wide spectrum of feeding strategies in southern hemisphere humpback whale baleen records. *PLoS ONE*. 2016;11(5):e0156698. https://doi.org/10.1371/journal.pone.0156698
29. Aguilar A, García-Vernet R. Fin whale, *Balaenoptera physalus*. In: Würsing B, Thewissen JGM, Kovacs KM, eds. *Encyclopedia of Marine Mammals*. San Diego, CA: Academic Press/Elsevier; 2017:368-371.
30. Ryan C, McHugh B, Trueman CN, et al. Stable isotope analysis of baleen reveals resource partitioning among sympatric rorquals and population structure in fin whales. *Mar Ecol Prog Ser*. 2013;479:251-261. https://doi.org/10.3354/meps10231
31. Wood SN. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J R Stat Soc (B)*. 2011;73(1):3-36. https://doi.org/10.1111/j.1467-9868.2010.00749.x
32. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2017 http://www.R-project.org.
33. Schwert M, Auerswald K, Schnyder H. Reconstruction of the isotopic history of animal diets by hair segmental analysis. *Rapid Commun Mass Spectrom*. 2003;17:1312-1318. https://doi.org/10.1002/rcm.1042
34. Grecian WJ, McGill RAR, Phillips RA, Ryan PG, Furness RW. Quantifying variation in $\delta^{13}C$ and $\delta^{15}N$ isotopes within and between feathers and individuals: is one sample enough? *Mar Biol*. 2015;162:733-741. https://doi.org/10.1007/s00227-015-2618-8
35. Cardona L, Vales D, Aguilar A, Crespo E, Zenteno L. Temporal variability in stable isotope ratios of C and N in the vibrissa of captive and wild adult South American sea lions *Otaria byronia*: more than just diet shifts. *Mar Mamm Sci*. 2017;33:975-990. https://doi.org/10.1111/mms.12415
36. Ohsumi S, Nishiwaki M, Hibiya T. Growth of fin whale in the North Pacific. *Sci Rep Whales Res Inst Tokyo* 158. 13:97-133.
37. Robins JP. Age studies in the female humpback whale, *Megaptera nodosa* (Bonnaterre) in east Australian Waters. *Mar Freshw Res* 1960;11(1):1–13. https://doi.org/10.1071/MF9600001.
38. Ruud JT. The surface structure of the baleen plates as a possible clue to age in whales. *Hvalrådets Skrifter*. 1940;23:1-24.
39. Kasuya T, Rice DW. Notes on baleen plates and on arrangement of parasitic barnacles of gray whale. *Sci Rep Whales Res Inst Tokyo*. 1970;22:39-43.
40. Sumich JL. Growth of baleen of a rehabilitating gray whale calf. *Aquat Mamm*. 2001;27(3):234-238.
41. Horwood J. Sei whale, *Balaenoptera borealis*. In: Würsing B, Thewissen JGM, Kovacs KM, eds. *Encyclopedia of Marine Mammals*. San Diego, CA: Academic Press/Elsevier; 2017:845-847.
42. Arnold PW, Birtles RA, Dunstan A, Lukoschek V, Matthews M. Colour patterns of the dwarf minke whale *Balaenoptera acutorostrata* sensu lato: description, cladistic analysis and taxonomic implications. *Memoirs Queensland Mus*. 2005;51:277-307.
43. Cerchio S, Yamada TK. Omura's whale, *Balaenoptera omurai*. In: Würsing B, Thewissen JGM, Kovacs KM, eds. *Encyclopedia of Marine Mammals*. San Diego, CA: Academic Press/Elsevier; 2017:656-659.
44. Tershy BR, Wiley DN. Asymmetrical pigmentation in the fin whale: a test of two feeding related hypotheses. *Mar Mamm Sci*. 1992;8(3):315-318. https://doi.org/10.1111/j.1748-7692.1992.tb00416.x
45. Arregui M, Josa M, Aguilar A, Borrell A. Isotopic homogeneity throughout the skin in small cetaceans. *Rapid Commun Mass Spectrom*. 2017;31:1551-1557. https://doi.org/10.1002/rcm.7936
46. Williams TM, Dunkin R, Yochem P, et al. Assessing stable isotope signature variation in cetaceans: an evaluation of skin sampling techniques and correlations with diet for bottlenose dolphins and killer whales. NWFSC Contract Report; 2008.
47. Minagawa M. Reconstruction of human diet from $\delta^{13}C$ and $\delta^{15}N$ in contemporary Japanese hair: a stochastic method for estimating multi-source contribution by double isotopic tracers. *Appl Geochem*. 1992;7:145-158. https://doi.org/10.1016/0883-2927(92)90033-Y
48. O'Connell TT, Hedges REM. Investigations into the effect of diet on modern human hair isotopic values. *Am J Phys Anthropol*. 1999;108:409-425. https://doi.org/10.1002/(SICI)1096-8644(199904)108:4<409::AID-AJPA3>3.0.CO;2-E
49. Mitchell E. Whale pigmentation and feeding behavior. *Am Zool*. 1972;12:655.
50. Caro T, Beeman K, Stankowich T, Whitehead H. The functional significance of colouration in cetaceans. *Evol Ecol*. 2011;25:1231-1245. https://doi.org/10.1007/s10682-011-9479-5

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: García-Vernet R, Sant P, Víkingsson G, Borrell A, Aguilar A. 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*. 2018;32:1257-1262. https://doi.org/10.1002/rcm.8169

Sulfur stable isotope ratios provide further insight into movements of the fin whale, an oceanic long-range migrant

Autores: **Raquel García-Vernet***[1], Alex Aguilar[1], Julia Zafra[1], Gísli Víkingsson[2], Sverrir Daniel Halldórsson[2], Asunción Borrell[1]

[a] *Departamento de Biología Evolutiva, Ecología i Ciencias Ambientales, y IRBio, Facultad de Biología, Universidad de Barcelona*

[b] *Marine and Freshwater Research Institute, Fornubúðum 5, 220 Hafnarfjörður, Iceland*

Stable isotope ratios have proven a valuable tool to investigate marine mammal ecology, including diet of species, distribution and migratory movements. While most studies have focused on δ15N and δ13C values, δ34S values have been little used because their pattern of variation and tissue dynamics remain unclear. We examined the sequential variation of δ15N, δ13C and δ34S values along the baleen plates from fin whales occurring off West Iceland in summer. All baleen plates exhibited fluctuations along their growing axis. A significant synchronic correlation was found between δ15N and δ34S values, while the relation of these values with δ13C was highly variable and inconsistent. These results were similar to those obtained in previous studies in Greenland bowhead whales, although in fin whales the pattern of the oscillations showed an increase in values during winter as opposed to bowhead whales, which showed a decrease. Although seasonal variations in food intake and the associated cycles of protein synthesis and catabolism may have played a role in such fluctuations and the observed differences between species, we suggest that the main driver for the δ34S fluctuations reflected in baleen plates is the variation of local baselines between winter and summer grounds. This suggests ample potential for using δ34S values to study migratory movements and destinations of marine megafauna provided that the geographic variation in δ34S baselines are clarified.

Sulfur stable isotope ratios provide further insight into movements of the fin whale, an oceanic long-range migrant

Raquel García-Vernet*[1], Alex Aguilar[1], Julia Zafra[1], Gísli Víkingsson[2], Sverrir Daniel Halldórsson[2], Asunción Borrell[1]

[1]*Department of Evolutionary Biology, Ecology and Environmental Sciences, and IRBio, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain*

[2]*Marine and Freshwater Research Institute, PO Box 1390, Fornubúðum 5, 220, Hafnarfjörður, Iceland*

*Correspondence: Raquel García-Vernet (Telephone number: + 34 93 402 1453; Barcelona, Spain).

Email: rakel.8592@gmail.com

ABSTRACT

Stable isotope ratios have proven a valuable tool to investigate marine mammal ecology, including diet of species, distribution and migratory movements. While most studies have focused on $\delta^{15}N$ and $\delta^{13}C$ values, $\delta^{34}S$ values have been little used because their pattern of variation and tissue dynamics remain unclear. We examined the sequential variation of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values along the baleen plates from fin whales occurring off West Iceland in summer. All baleen plates exhibited fluctuations along their growing axis. A significant synchronic correlation was found between $\delta^{15}N$ and $\delta^{34}S$ values, while the relation of these values with $\delta^{13}C$ was highly variable and inconsistent. These results were similar to those obtained in previous studies in Greenland bowhead whales, although in fin whales the pattern of the oscillations showed an increase in values during winter as opposed to bowhead whales, which showed a decrease. Although seasonal variations in food intake and the associated cycles of protein synthesis and catabolism may have played a role in such fluctuations and the observed differences between species, we suggest that the main driver for the $\delta^{34}S$ fluctuations reflected in baleen plates is the variation of local baselines between winter and summer grounds. This suggests ample potential for using $\delta^{34}S$ values to study migratory movements and destinations of marine megafauna provided that the geographic variation in $\delta^{34}S$ baselines are clarified.

Keywords: baleen whale, Iceland, Stable isotope covariation, baleen plate, migration, nitrogen, carbon, sulfur

1. INTRODUCTION

Stable isotope analyses have proven particularly useful to investigate trophic ecology of animals that are difficult to observe and study, such as baleen whales and other marine mammals (Newsome et al. 2010; Ramos & González-Solís 2012). Most studies in these species have focused on nitrogen and carbon stable isotopes, which are sensible to spatial and trophic variations (Newsome et al. 2010). Thus, the stable isotope ratio of nitrogen ($^{15}N/^{14}N$), denoted by the $\delta^{15}N$ value, increases between 2 – 4‰ at each trophic step and therefore reflects the position of organisms in the food web (Caut et al. 2009). However, it is also affected by other factors, such as spatial distribution (McMahon et al. 2013), nutritional condition (Hobson et al. 1993), or feeding on a protein rich diet (Florin et al., 2011). In turn, the stable isotope ratio of carbon ($^{13}C/^{12}C$), denoted by the $\delta^{13}C$ value, although it may also be influenced to some extent by trophic level (e.g. Caut et al, 2009), it mostly reflects that of primary producers; in marine habitats, low $\delta^{13}C$ values are associated to pelagic habitats, and high values to benthic and coastal habitats (Peterson & Fry 1987; Hobson et al. 1994; Hobson 1999). As a result, the combined use of $\delta^{15}N$ and $\delta^{13}C$ values have been widely used to infer diet (e.g., Marcoux et al. 2012, Lauriano et al. 2020), movement patterns (e.g., Matthews & Ferguson 2015), or trophic niche partitioning between species or populations of marine mammals (e.g. Gavrilchuk et al. 2014; Witteveen & Wynne 2016).

In recent years, stable isotopes of other elements have increasingly received attention for their ability to provide complementary information to that provided by $\delta^{15}N$ and $\delta^{13}C$ values. In particular, the stable isotope ratio of sulfur ($^{34}S/^{32}S$), denoted by the $\delta^{34}S$ value, has been occasionally applied to infer habitat use. Although $\delta^{34}S$ values of seawater appear to vary little geographically, those for marine sulfate are generally higher than those in terrestrial systems, similarly as it occurs with $\delta^{13}C$ values (Lott et al. 2003). Therefore, in studies of marine mammal ecology, the application of $\delta^{34}S$ values has focused in distinguishing between individuals feeding inshore and those feeding offshore, but not in assessing oceanic-scale movements (Niño-Torres et al. 2006; MacAvoy et al. 2015; Borrell et al. 2021; García-Vernet et al. 2021). Some studies have found correlations between $\delta^{15}N$ and $\delta^{34}S$ values (Matthews & Ferguson 2015) as well as a $\delta^{34}S$ trophic increase (McCutchan et al. 2003), and this has been taken as an indication that $\delta^{34}S$ values may be affected to some degree by factors other than the ecosystem baselines, thus adding further complexity to the use of $\delta^{34}S$ values.

Baleen plates are particularly useful for studying the covariation over time of the stable isotope ratios of different elements. Baleen grow continuously but, once the tissue that

makes up the plate is deposited in the gum, it becomes biologically inert and thereafter its composition remains stable. The continued deposition of baleen tissue produces a layering that provides a sequential record of the body pool values at the various moments in which the tissue was deposited. Taking advantage of this property, analyses of $\delta^{13}C$ and $\delta^{15}N$ values in sequential layers of the baleen have been widely used to infer migratory patterns and shifts in diet of mysticetes (Caraveo-Patiño et al. 2007; Bentaleb et al. 2011; Matthews & Ferguson 2015; Aguilar et al. 2014; Eisenmann et al. 2016; Reiss et al. 2020).

However, the only study so far published examining covariation of $\delta^{34}S$ values with those of $\delta^{15}N$ and $\delta^{13}C$ is that conducted by Matthews & Ferguson (2015) in three bowhead whales (*Balaena mysticetus*) from Greenland. These authors found a positive correlation between $\delta^{34}S$ and $\delta^{15}N$ which they attributed to the fact that $\delta^{34}S$ values may be affected not only by environmental baselines but possibly also by a decline in food consumption during winter.

The fin whale (*Balaenoptera physalus*) is a highly migratory species with an oceanic distribution. It feeds intensively during the summer and severely reduces its food intake during winter (Aguilar and García-Vernet, 2018). In the North Atlantic, one of its main summer feeding grounds is located off western Iceland (Víkingsson et al., 2009). The fact that Iceland has a high concentration of active volcanoes due to its position on the Mid-Atlantic Ridge and that the $\delta^{34}S$ values of volcanic emissions is lower than the mean oceanic water values (Strauss 2004), allows us to constrain interpretations of variation in $\delta^{34}S$ values along the baleen plates of fin whales feeding there. In this context, we predicted: 1) If $\delta^{34}S$ variation is caused by the decrease of fractionation due to food scarcity, winter values would be lower and correlated with $\delta^{15}N$ values, whereas 2) if $\delta^{34}S$ variation is related to migratory geographical shifts (*i.e.* baselines), then $\delta^{34}S$ values would be lower in summer because of the influence of volcanism and not necessarily correlated with $\delta^{15}N$ or $\delta^{13}C$ values.

2. MATERIALS AND METHODS

2.1. Sample collection

Baleen plates were obtained from ten fin whales caught off south-western Iceland and flensed at the Hvalur H/F whaling station (Hvalfjörður) in 2013 (n=5) and 2015 (n=5). Export/import licenses under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) were obtained for this material from the

respective Icelandic and Spanish authorities (permit numbers are: 15IS017MA and ESBB00207/15I, respectively).

To embrace the longest possible record, baleen plates were sampled from the mid-posterior part of the baleen rack, where the largest and less eroded baleen stand. Baleen plates differ in size in different positions of the mouth, but this difference does not affect the stable isotope ratios oscillations because baleen growth rates are constant along the baleen rack (García-Vernet et al. 2018; Aguilar & Borrell 2021). Baleen were excised complete from the gum and preserved at -20ºC. Previous studies had shown that gum has different stable isotope ratios than those of the baleen plate surface (Rita et al. 2019), so the gum tissue was removed using a cutter. Any remaining adhered materials were removed with steel wool, and the surface of the plate was cleaned using a (2:1) chloroform-methanol solution. Once cleaned, baleen plates were stored dry until the analysis.

2.2. Sampling and stable isotope analysis

Subsamples were extracted sequentially along the growth axis of the baleen using a grinder at every centimeter starting from the proximal end of the baleen (that most recently formed) to the most distal (that oldest formed). 30 points were subsampled from each baleen plate (Fig. 1).

For conducting $\delta^{15}N$ and $\delta^{13}C$ analyses, approximately 0.3 mg of each of the powdered subsamples were weighed into tin capsules. Subsamples were loaded and combusted at 1000°C to be analyzed using a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Flash 1112 elemental analyzer; CE Elantech, Lakewood, NJ, USA), coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (both from ThermoFinnigan, Bremen, Germany).

For conducting $\delta^{34}S$ analyses, approximately 1mg of each of the powdered subsamples were weighed into tin capsules. Subsamples were loaded and combusted at 1030ºC and analyzed with an Elemental Analyzer (Carlo Erba 1108) coupled to a Delta Plus xp isotope ratio mass spectrometer via a ConFlow III interface (both from Thermofisher).

International isotope secondary standards of known $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ ratios in relation to Vienna Pee Dee Belemnite (V-PDB) calcium carbonate, atmospheric nitrogen (AIR) and Vienna-Canyon Diablo Troilite (VCDT)) respectively were used to calibrate the system and compensate for any analytical drift over time; namely: polyethylene (IAEA-CH-7; $\delta^{13}C = -31.8‰$), sucrose (IAEA-CH-6; $\delta^{13}C = -10.4‰$), ammonium sulfate (IAEA-

N-1; $\delta^{15}N$ = +0.4‰ and IAEA-N-2; $\delta^{15}N$ = +20.3‰), potassium nitrate (USGS34; $\delta^{15}N$= −1.7‰), L-glutamic acid (USGS40; $\delta^{15}N$= −4.6‰; δ13C= −26.2‰) and caffeine (IAEA-600; $\delta^{15}N$ = 1.0‰; $\delta^{13}C$= −27.7‰), barium sulfate (NBS127 $\delta^{34}S$ = +20.3 and IAEA-SO-6 $\delta^{34}S$ = -34.1), barium sulfate salt (IAEA-SO-5; $\delta^{34}S$ = +0.5) and YCEM ($\delta^{34}S$= +12.8. Analytical precision for repeat measurements of the reference material, run in parallel with the baleen subsamples, was 0.3‰ for $\delta^{13}C$, 0.1‰ for $\delta^{15}N$, and 0.1‰ for $\delta^{34}S$ (1 SD, n = 10).

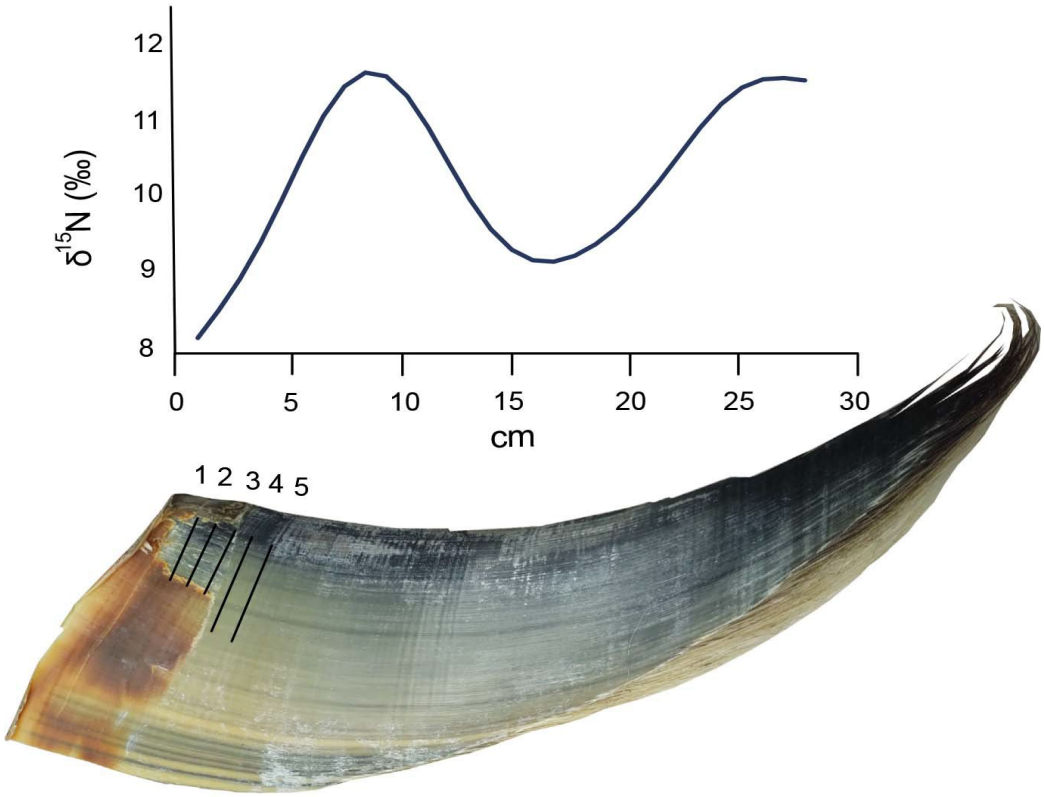


Figure 1*: Schematic figure showing the subsampling proceedings. At the bottom, a baleen plate with the first 5 subsampling points, spaced at 1 cm intervals, is shown. The left part of the baleen plate reflects the most recently synthetized tissue, which corresponds to the signal acquired in Icelandic waters. At the top of the figure, there is an example of the expected isotopic record after analyzing subsamples extracted along the growth axis of the baleen.*

All reference materials used for the analysis were selected according to previous calibration experiments performed on the same type of tissue to ensure that the range of the reference values spanned those of the subsamples.

2.3. Statistical analysis

Mean and standard deviations were calculated for each baleen plate and isotope ratio. A generalized additive model (GAM) was fitted to the delta values of C, N and S for each individual. Each model was fitted to the results from each baleen plate by considering the delta values of a given element as the dependent variable, and the length of the plate as the independent variable. For each baleen plate and delta value, homoscedasticity and normality of the residuals were checked using gam.check. To test whether the basis dimension for a smooth was adequate to our data, we checked the k-index and p.value, also provided by the gam.check function. Then, models were adjusted by removing outliers and choosing the best k for each model. Parameters estimated for each model can be found in TableS1. Finally, we used the GAM fitted to the $\delta^{15}N$ values to estimate the baleen plate growth rates occurring during a year (complete cycle), calculated as the distance between two sequential maximum peaks (Aguilar et al. 2014).

To assess the correlations among the three stable isotope ratios in the same individual, cross correlation functions (CCF) were calculated and plotted for the different combinations of isotope ratios in each baleen plate. Cross correlation functions between two variables are useful to identify the lags in which the first variable shows higher correlations with the second variable. All the statistical analyses were performed using R version 4.0.2.

3. RESULTS

We obtained $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values from 30 sampling sites from each baleen, which totaled 300 subsamples. All baleen plates exhibited fluctuations along their growing axis, but these fluctuations presented variability among individuals. The magnitude of variation for $\delta^{15}N$ values along baleen plates was higher than for those of $\delta^{13}C$ and $\delta^{34}S$ values (Fig 2, Table 1).

Distance between two maximum $\delta^{15}N$ values showed consistence among individual's oscillations, with one cycle being completed every 14-19 cm (mean = 16.8 cm, SD = 1.5 cm). However, two individuals presented different patterns: F15076 did not show clear oscillations and the distance between $\delta^{15}N$ peaks could not be determined, while F15080 presented clearer oscillations but spacing between maximum $\delta^{15}N$ values was shorter than for the other plates (Fig 2) probably due to differences in behavior and not to a differential growth rate (see Aguilar et al. 2014). As a consequence, these two individuals were not included in the calculations of the mean cycle.

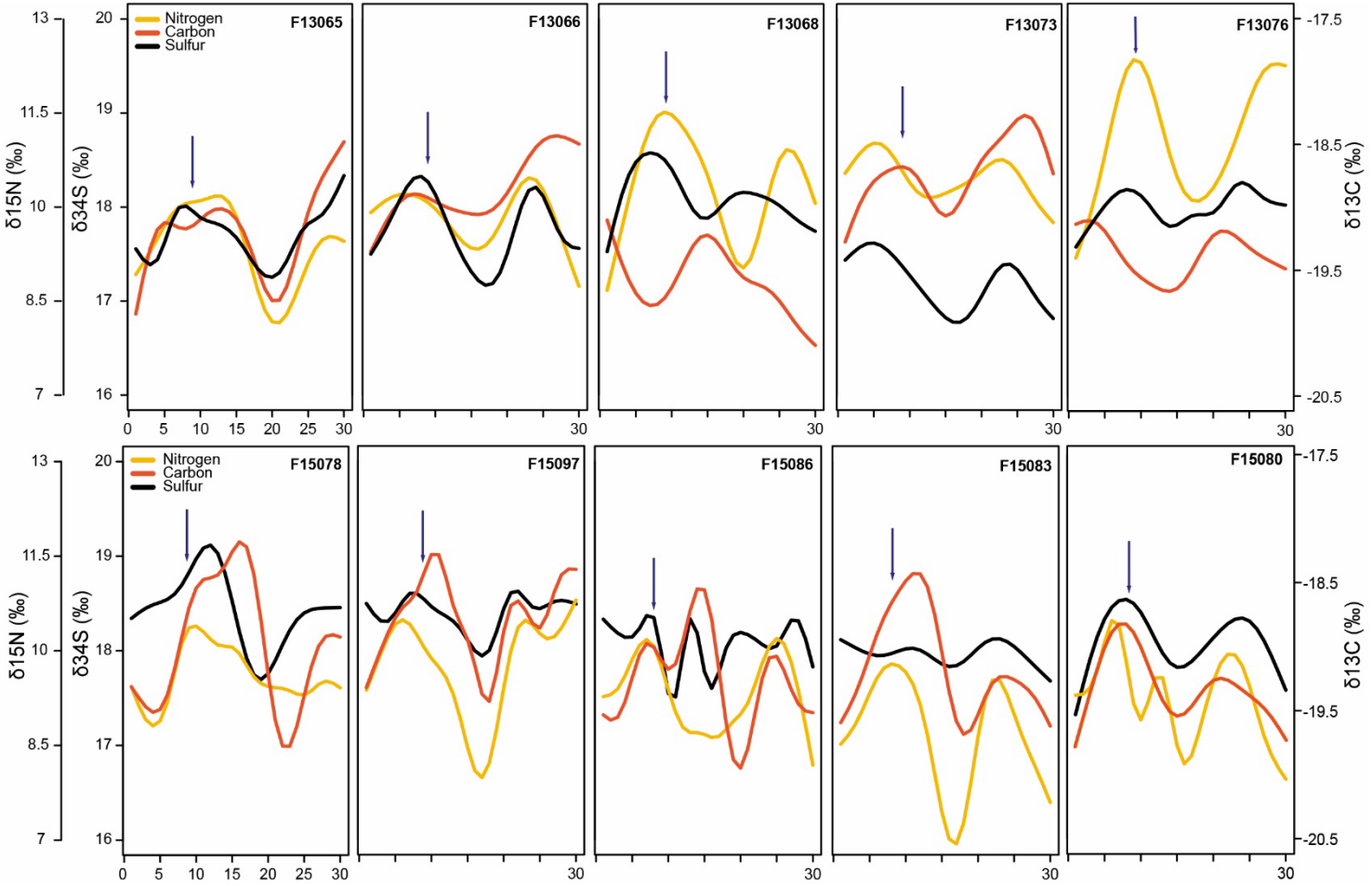


***Figure 2**: Results of the GAM fitted for each baleen plate for all the stable isotopes analyzed along the growing axis of the plates. Blue arrows indicate the position of cm 8, which approximately corresponds to the winter season (half of the annual cycle)*

The $\delta^{15}N$ and $\delta^{34}S$ values followed strikingly similar oscillations. Values of the most proximal part of the baleen plates, that is, the one most recently synthetized and which purportedly reflects the isotopic ratios acquired in Icelandic waters during the summer, were in most cases the lowest of the series. $\delta^{15}N$ and $\delta^{34}S$ values increased thereafter, until reaching a peak in approximately the mid-position of the first oscillation period, which purportedly reflects stable isotope ratios acquired during the winter season (see results above). Thereafter, both isotopic ratios declined again to reach a second minimum, purportedly corresponding to the previous summer. $\delta^{13}C$ patterns were not so regular; they varied greatly among individuals, but they also fluctuated between low values in summer and high values in winter.

Table 1: *Minimum and maximum delta values obtained along the axis of each baleen plate. In addition, for each baleen plate, means and standard deviations for each stable isotope were calculated.*

	$\delta^{15}N$ values		**$\delta^{13}C$ values**		**$\delta^{34}S$ values**	
	Min - max (‰)	**Mean (‰)**	**Min - max (‰)**	**Mean (‰)**	**Min - max (‰)**	**Mean (‰)**
F13065	8.0 – 10.2	9.4 ± 0.7	-19.8 – -18.4	-19.2 ± 0.3	17.1 – 18.4	17.7 ± 0.3
F13066	8.6 – 10.5	9.8 ± 0.5	-19.3 – -18.4	-18.9 ± 0.3	17.0 – 18.4	17.8 ± 0.4
F13068	8.6 – 11.7	10.3 ± 0.9	-20.2 – -19.0	-19.6 ± 0.3	17.4 – 18.6	18.1 ± 0.3
F13073	9.8 – 11.1	10.5 ± 0.3	-19.3 – -18.1	-18.7 ± 0.3	16.1 – 17.7	17.2 ± 0.4
F13076	9.3 – 12.4	11.1 ± 1.0	-19.7 – -19.0	-19.4 ± 0.2	17.6 – 18.3	18.0 ± 0.2
F15078	8.7 – 10.5	9.6 ± 0.5	-19.8 – -18.2	-19.0 ± 0.5	17.6 – 19.2	18.4 ± 0.4
F15097	7.5 – 10.9	9.8 ± 0.8	-19.0 – -18.2	-18.7 ± 0.3	17.8 – 18.8	18.4 ± 0.2
F15086	7.9 – 10.5	9.3 ± 0.7	-20.1 – -18.2	-19.2 ± 0.4	17.3 – 18.4	18.1 ± 0.3
F15083	5.4 – 10.0	8.7 ± 1.1	-19.9 – -18.3	-19.1 ± 0.5	17.5 – 18.4	18.0 ± 0.2
F15080	7.5 – 10.3	9.2 ± 0.8	-20.4 – -18.7	-19.4 ± 0.4	17.2 – 18.5	18.0 ± 0.4

Significant correlations were found among the different stable isotope ratios in the same baleen plate, reflecting synchronic patterns (Table 2). It is noteworthy that $\delta^{34}S$ and $\delta^{15}N$ values along each baleen plate showed significant and positive correlations at lag 0 in the 10 individuals analyzed (Fig 3).

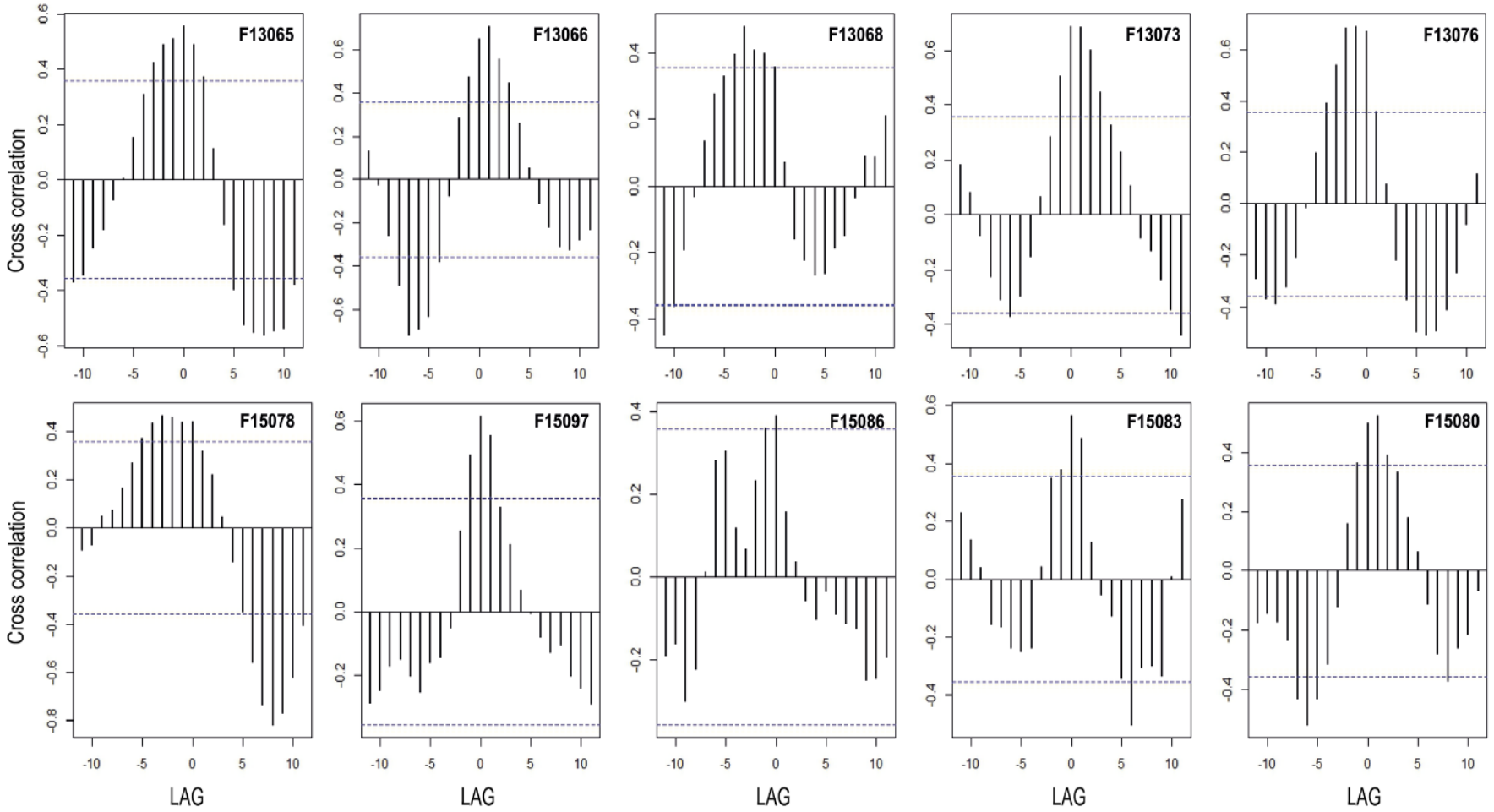


***Figure 3**: Results of the Cross-correlation functions, in which we compared the variation in the $\delta^{34}S$ and $\delta^{15}N$ values determined in the seriated subsampling points from the ten baleen plates analyzed. Dotted lines indicate the significance level.*

***Table 2**: Number of baleen plates that showed significant correlation in their $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values determined in the sampling points along the baleen plates.*

	$\delta^{15}N$		**$\delta^{34}S$**	
	Positive corr	Negative corr	Positive corr	Negative corr
$\delta^{34}S$	**10**	**0**		
$\delta^{13}C$	**4**	**1**	**3**	**0**

On the other hand, correlations among $\delta^{13}C$ values and the other stable isotope ratios presented larger variability (Fig S1, Fig S2) than between $\delta^{15}N$ and $\delta^{34}S$ values. $\delta^{13}C$ and $\delta^{15}N$ values significantly correlated at lag 0 in 5 out of 10 baleen plates (F13065, F13076, F15078, F15097, F15083), and in one case (F13076) the correlation was negative. $\delta^{13}C$ and $\delta^{34}S$ values presented positive and significant correlations in 3 out of 10 baleen plates (F13065, F15097, F15080), although in other individuals there were still some patterns that could be discerned visually despite results in CCF being non-significant (see for example F15078).

4. DISCUSSION

Baleen plates exhibited fluctuations along their growing axis for all the studied stable isotope ratios. This is a common result in studies of this type conducted on baleen plates and the fluctuations have been traditionally attributed either to migratory movements that implied crossing different isotopic baselines, to seasonal variation in feeding patterns and diet composition, or to a combination of both (e,g. Caraveo-Patiño et al. 2007; Aguilar et al. 2014, Matthews & Ferguson 2015, Eisenmann et al. 2016, Eerkes-Medrano et al. 2021). The amplitude and period of the fluctuations differed among individuals, undoubtedly reflecting variation in migration pattern and/or feeding behavior among individuals. The mean annual growth of baleen plates analyzed in this study was estimated at 16.8 cm (SD= 1.5), value which is consistent with previous studies in fin whales (Bentaleb et al. 2011, Aguilar et al. 2014, Riekenberg et al. 2021).

Fluctuations of $\delta^{13}C$ values were little pronounced and extremely variable among individuals. In most cases they were not correlated with those of $\delta^{34}S$ or $\delta^{15}N$ values and, in the few cases in which they were, the direction was not always the same. At least two individuals showed a clear decrease in $\delta^{13}C$ values during the winter season, while the remaining individuals either did not show a clear pattern or an increase in $\delta^{13}C$ values during the summer season. This made interpretation of results difficult. It is known that

$\delta^{13}C$ values are related to proximity to the coast and, to some extent, also to trophic transfer which implies a slight increment of values when moving up through the food web (Peterson & Fry 1987). At oceanic scale, $\delta^{13}C$ value baselines also exhibit latitudinal gradients (McMahon et al. 2013), a fact that has been successfully applied to investigate migration of right whales in the southern hemisphere (Best and Schell 1996). However, the gradient of $\delta^{13}C$ variation in the northern hemisphere is much lower than in the southern hemisphere (McMahon et al. 2013), and it has been suggested that the lack of marked oscillations in baleen plates of northern hemisphere minke whales could be a consequence of that (Mitani et al. 2006). Starvation may also affect $\delta^{13}C$ values, but the underlying mechanisms seem to be complex and still not fully understood (Doi et al. 2017). During fasting, both muscle protein and adipose tissue can be used as carbon source; while muscle protein will produce ^{13}C enrichment in the body's carbon pool, the use of adipose tissue as carbon source will produce the opposite effect, leading to ^{13}C depletion (Polischuk et al. 2001). This complex scenario makes it difficult to use $\delta^{13}C$ values as indicators of fasting in the baleen plate records (Ishikawa et al. 2021). Although we are not able to identify the specific mechanisms that drive variation in $\delta^{13}C$ fluctuation of baleen plates, we attribute them to heterogeneities in the migratory and feeding behavior of the fin whales that visit Icelandic waters.

Conversely, fluctuations in $\delta^{15}N$ values were clear and quite consistent among individuals. Values tended to be low in summer, when the samples had been collected, and to increase during autumn and winter, confirming previous studies conducted on this population (García-Vernet et al. 2018). Like most mysticetes, fin whales alternate high-latitude summer grounds, where productivity is high and permits intensive feeding, with lower latitude winter grounds, where food is much scarcer and whales experience lower food intake or are even forced to fast, but where water temperatures are warmer and more suitable for calving (Aguilar & García-Vernet 2018). Taking such a migratory regime into account, oscillations in δ15N values would be likely reflecting varying local baselines potentially combined with variations in diet composition between seasons, as the stable isotopic ratio of Nitrogen is strongly affected by trophic level (Peterson and Fry 1987; Kelly 2000). The relative contribution of these two sources of variation on the oscillations observed is difficult to discern, but it is highly likely that they result from a combination of both factors.

$\delta^{34}S$ values were positively correlated with those of $\delta^{15}N$ in all baleen plates, in concordance with results obtained in a previous study in bowhead whales (Matthews & Ferguson, 2015). Matthews & Ferguson (2015) argued that the observed correlation

could be mainly due to 2 reasons: i) decreased metabolic activity that affects both stable isotopic ratios, and ii) a parallel covariation in the baselines of these two ratios in the various grounds visited by the whales. The relative contribution of these two effects, if existing, was impossible to disclose.

In general, $\delta^{34}S$ values show slight differences among tissues (Arneson & MacAvoy 2005, Webb et al. 2017) and their discrimination between diet and the consumer tissues is considered negligible (see for example Krajcarz et al. 2021). This appears confirmed by the fact that different baleen whale species sharing a common feeding ground, but exploiting different trophic levels, show very similar $\delta^{34}S$ values (García-Vernet et al. 2021). All this combined supports the idea that $\delta^{34}S$ values do not depend on trophic position. However, some studies point to the opposite direction and indeed show the existence of some degree of $\delta^{34}S$ trophic fractionation. If this process actually takes place, trophic level and, to some degree, the protein content of the diet (McCutchan et al. 2003) would be affecting similarly $\delta^{34}S$ and $\delta^{15}N$ and would thus induce positive covariation. The apparent reason why trophic level has a greater effect on $\delta^{15}N$ values than on $\delta^{34}S$ values is that fractionation in the former is affected by the metabolism of several amino acids, while in the latter it would be affected by the metabolism of two of them, methionine and cysteine, the two sulfur amino acids that are incorporated into proteins. Moreover, methionine is an essential amino acid, and therefore does not typically participate in metabolic reactions involving fractionation, and cysteine is semi-essential but it is in its turn synthetized from methionine (Brosnan & Brosnan 2006; Florin et al. 2011). All this strongly limits the scope of the fractionation of $\delta^{34}S$ values, a process that would only occur under certain circumstances, thus mitigating the effect of shifts in trophic level or protein intake.

Baleen plates are mainly composed of amorphous keratin and alpha-keratin (Aubin et al. 1984), and the vast majority of the sulfur present in this protein comes from cysteine (Wang et al. 2016). Cysteine is synthesized by deriving sulfur from methionine (Finkelstein 1990), Cysteine, in turn, is the precursor of other sulfur-containing organic molecules, and ^{34}S fractionation seems to occur with cysteine catabolism (Tanz & Schmidt 2010). In rats and humans, the catabolism of methionine and cysteine appears to be restricted to situations of low food intake (Storch et al. 1988, 1990, Stipanuk et al. 1992). Thus, in periods of limited protein intake, $\delta^{34}S$ discrimination from diet to keratin would presumably reduce, and lead to lower $\delta^{34}S$ values than when methionine and cysteine are adequately supplied and easily catabolized (Matthews & Ferguson 2015). According to these mechanisms, in the fin whales here studied we should have expected

lower $\delta^{34}S$ values during the winter period of food scarcity, as Matthews and Ferguson (2015) observed in bowhead whales.

However, our results match exactly the opposite pattern, with clear $\delta^{34}S$ peaks in those segments of the baleen plate that were synthetized in winter, and lower values in segments synthetized in summer. This would suggest that the recycling of proteins, promoted by fasting or by a lowered consumption of protein, may indeed increase the $\delta^{34}S$ values of the body pool, as suggested by Richards et al. (2003). These authors found an unusual ^{34}S enrichment between low-protein diet and the hair of horses, which they suggested was the result of a higher contribution to cysteine synthesis of endogenous methionine sulfur, which would be ^{34}S enriched compared to diet. Although it is likely that in baleen whales sparse winter foraging and metabolic adaptations may come into play to inactivate massive protein catabolism during winter (Aguilar et al. 2014), it cannot be excluded that some degree of protein recycling occurs. This might have caused the observed increase of $\delta^{34}S$ observed in the Icelandic fin whales. If that were the case, the winter increase of $\delta^{34}S$ values in fin whales would be consistent with the findings of Richards et al. (2003). Its lower magnitude and variability among individuals as compared to that of $\delta^{15}N$ value would be explained by the former only reflecting methionine sulfur recycling, while the latter reflecting the recycling of nitrogen from several amino acids.

Whatever the case, if $\delta^{34}S$ is indeed affected by food intake and/or by protein catabolism, and independently of the consequences of such effect, the question remains open as to why the variation of the $\delta^{34}S$ values along the baleen plate is different between bowhead and fin whales, two species that have traditionally been assumed to endure fasting during winter or, at least, to be subject to stringent seasonal variations in food intake (Lowry & Frost 1984; Aguilar & García-Vernet, 2018). A main difficulty to answer this question it that the actual magnitude of the seasonal variation in food intake occurring in the two species, and its actual incidence on the body pool of the various elements and their stable isotope ratios, still remains unclear (Aguilar et al. 2014; Pomerleau et al. 2018, Fortune et al. 2020). Once this is clarified, it will be possible to ascertain the actual effect that such variations would have on stable isotope ratios, particularly those of sulfur.

Baseline variation is a second plausible explanation for the observed fluctuations in $\delta^{34}S$ values. This hypothesis was also contemplated by Matthews and Ferguson (2015) in their study on bowhead whales. They suggested that the Hudson Bay and Hudson Strait environments, where bowheads overwinter, may be relatively depleted in ^{34}S, and this would explain the decrease in $\delta^{34}S$ values found in the baleen plate during winter. In the

open waters of the oceans, $\delta^{34}S$ values of dissolved sulfates lie around 21‰ and are generally accepted to be geographically very homogenous (Rees et al. 1978). Riverine inputs are the main source of sulfates into the ocean, and they have $\delta^{34}S$ values around 8‰, but the range of their effect appears quite limited (Strauss 2004). As a result, the differences between river and oceanic values have given use to the isotope ratios of sulfur as proxies for determining inshore – offshore movements or distribution (Niño-Torres et al. 2006; MacAvoy et al. 2015; Borrell et al. 2021; García-Vernet et al. 2021). However, fin whales tend to remain outside the continental slope (Aguilar & García-Vernet 2018) so they shouldn't be susceptible to the effect of riverine inputs. Moreover, the lack of correlation between $\delta^{34}S$ and $\delta^{13}C$ values in the baleen plates supports the hypothesis that distance to the coast is not a main driver of the fluctuations in $\delta^{34}S$ values observed here.

Another source of sulfur into the oceans are the undersea volcanic mountains that extend along the mid ocean ridges. The $\delta^{34}S$ values of their emissions usually range between 3 and 13‰, and those associated to their volcanic activity range between -10 and 10‰ (Strauss 2004). Iceland is on the Mid-Atlantic Ridge and located in a tectonic and volcanic hotspot (Einarsson 1991), and the $\delta^{34}S$ values of its geothermal wells vary between −2.0 and +3.8‰, values that are extremely low as compared to those from other regions (Marini et al. 2011). Unfortunately, seascapes of stable isotope ratios similar to those published for other elements (e.g. McMahon et al. 2013) are not available for the North Atlantic, but a potential explanation to justify the low $\delta^{34}S$ values observed in the segments of the baleen plates that correspond to the periods when the fin whales occupy waters near Iceland would be the local emissions, with characteristic depleted values of volcanic origin. To our knowledge, no similar studies have been conducted with other migrant species from Iceland. However, $\delta^{34}S$ values in skin from fin whales summering in Iceland (García-Vernet et al. 2021) were slightly ^{34}S depleted compared to those from Mediterranean fin whales (Borrell et al. 2021): 18.8‰ ± 0.4 vs 19.3‰ ± 0.4, respectively. Such difference is statistically significant and would support the hypothesis of lower $\delta^{34}S$ values in waters surrounding Iceland as compared to other regions.

Still, a further explanation that should be considered is the relative contribution in the different water masses of the sulfur from anthropogenic sources (fossil fuel combustion as well as emissions of SO_2 and sulphate), which is typically characterized by relatively low $\delta^{34}S$ values, and that from marine biogenic sources, which is characterized by relatively high $\delta^{34}S$ values (Lin et al. 2012). It can be speculated that the variation observed along the baleen plates of fin whales may reflect the movement of the

individuals from a summer environment located close to anthropogenic sources (Iceland), to a winter environment located far away from these (the mid North Atlantic).

Adding to the restricted use that sulfur isotope ratios have had in the past to assess inshore-offshore movements, our results indicate that they may be useful indicators of the long-range movements and migrations of megafauna at oceanic scales. In particular, inert tissues with continuous growth, such as baleen plates whiskers, nails, carapace scutes, teeth or feathers (Ramos and González-Solís, 2012), may be promising for successfully detecting relatively low variations in isotopic patterns, as seen here for $\delta^{34}S$ and $\delta^{13}C$ values. However, with the present level of information we cannot apportion the relative contribution of the various potential effects here adduced to explain the variation observed in the $\delta^{34}S$ values along the baleen plates of fin whales, nor even establish whether they indeed have an actual effect. Further studies are needed to establish more accurately the effect of trophic level and protein intake on the $\delta^{34}S$ values of whale tissues and, particularly, on the actual patterns of geographic variation of such values in the environment.

ACKNOWLEDGMENTS

This study was part of project PID2020-119712GB-I00 funded by the Ministerio de Ciencia e Innovación of Spain, MCIN/AEI/10.13039/501100011033. R.G-V's work was funded through a Ph.D. APIF scholarship granted by the University of Barcelona. Authors are grateful to Hvalur H/F for the facilities provided to conduct the sampling, and to the staff of the MFRI for their collaboration during the fieldwork. The stable isotope analyses were conducted at the Centres Científics i Tecnològics (CCiT-UB) of the University of Barcelona. R.G-V wants to thank the Spanish State Public Employment Service (SEPE) for its financial support during the manuscript preparation process.

REFERENCES

Aguilar A, Borrell A (2021) Growth of baleen along the baleen rack is constant in balaenopterid whales. Polar Biol 44(6): 1223-1225. https://doi.org/10.1007/s00300-021-02877-6

Aguilar A, Giménez J, Gómez-Campos E, Cardona L, Borell A (2014) $\delta^{15}N$ value does not reflect fasting in mysticetes. *PLoS ONE* 9(3) e92288

Aguilar A, García-Vernet R (2018) Fin Whale. In W. Perrin F. Würsig B. Thewissen JGM. (Eds.) Encyclopedia of Marine Mammals (Third Edition): 368–371.

Arneson LS, MacAvoy SE (2005) Carbon, nitrogen, and sulfur diet–tissue discrimination in mouse tissues. Can J Zool 83(7): 989–995.

Aubin DJSt, Stinson RH, Geraci JR (1984) Aspects of the structure and composition of baleen, and some effects of exposure to petroleum hydrocarbons. Can J Zool 62(2): 193–198.

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.

Best PB, Schell DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar Biol 124: 483–494.

Borrell A, Gazo M, Aguilar A, Raga JA, Degollada E, Gozalbes P, García-Vernet R (2021) Niche partitioning amongst northwestern Mediterranean cetaceans using stable isotopes. Prog Oceanogr 193: 102559.

Brosnan JT, Brosnan ME (2006) The sulfur-containing amino acids: an overview. J Nutr 136(6): 1636S-1640S.

Caraveo-Patiño J, Hobson KA, Soto LA (2007) Feeding ecology of gray whales inferred from stable-carbon and nitrogen isotopic analysis of baleen plates. Hydrobiologia 586(1): 17–25.

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46: 443–453

Doi H, Akamatsu F, González AL (2017) Starvation effects on nitrogen and carbon stable isotopes of animals: an insight from meta-analysis of fasting experiments. R Soc open sci 4: 170633.

Eerkes-Medrano D, Aldridge DC, Blix AS (2021) North Atlantic minke whale (*Balaenoptera acutorostrata*) feeding habits and migrations evaluated by stable isotope analysis of baleen. Ecol Evol:16344-53.

Einarsson P (1991) Earthquakes and present-day tectonism in Iceland. Tectonophysics 189: 261-279

Eisenmann P, Fry B, Holyoake C, Coughran D, Nicol S, Nash SB (2016) Isotopic evidence of a wide spectrum of feeding strategies in Southern hemisphere humpback whale baleen records. PLoS ONE, 11(5): 1–20.

Finkelstein JD (1990) Methionine metabolism in mammals. J Nutr Biochem 1(5): 228–37.

Florin ST, Felicetti LA, Robbins CT (2011) The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. Funct Ecol 25(3): 519–526.

Fortune SM, Ferguson SH, Trites AW, LeBlanc B, LeMay V, Hudson JM, Baumgartner MF (2020) Seasonal diving and foraging behaviour of Eastern Canada-West Greenland bowhead whales. Mar Ecol Prog Ser 643: 197-217.

García-Vernet R, Sant P, Víkingsson G, 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: 1257–1262.

García-Vernet R, Borrell A, Vikingsson GA, Halldórsson SD, Aguilar A (2021) Ecological niche partitioning between baleen whales inhabiting Icelandic waters. Prog Oceanogr 199: 102690

Gavrilchuk K, Lesage V, Ramp C, Sears R, Bérubé M, Bearhop S, Beauplet G (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.

Gómez-Campos E, Borrell A, Aguilar A (2011) Nitrogen and carbon stable isotopes do not reflect nutritional condition in the striped dolphin. Rapid Commun Mass Spectrom 25(9): 1343-1347.

Hobson KA, Alisauskas RT, Clark RG (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. Condor 95: 388–394.

Hobson KA, Piatt JF, Pitocchelli J (1994) Using Stable Isotopes to Determine Seabird Trophic Relationships. J Anim Ecol 63(4): 786–799

Hobson KA (1999) Tracing origins and migration of widlife using stable isotopes: a review. *Oecologia* 120(3): 314–326.

Ishikawa H, Otsuki M, Tamura T, Konishi K, Bando T, Ishizuka M, Ikenaka Y, Nakayama SM, Mitani Y (2021) Foraging ecology of mature male Antarctic minke whales (*Balaenoptera bonaerensis*) revealed by stable isotope analysis of baleen plates. Polar Sci: 100785.

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 78(1): 1–27.

Krajcarz MT, Krajcarz M, Drucker DG, Bocherens H (2019) Prey-to-fox isotopic enrichment of 34S in bone collagen: Implications for paleoecological studies. Rapid Commun Mass Spectrom 33: 1311 – 1317.

Lauriano G, Pirotta E, Joyce T, Pitman RL, Borrell A, Panigada S (2020) Movements, diving behaviour and diet of type-C killer whales (*Orcinus orca*) in the Ross Sea, Antarctica. Aquat Conserv 30: 2428–2440.

Lin CT, Baker AR, Jickells TD, Kelly S, Lesworth T (2012) An assessment of the significance of sulphate sources over the Atlantic Ocean based on sulphur isotope data. Atmos Environ 62: 615-621.

Lott CA, Meehan TD, Heath JA (2003) Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: Influence of marine prey base. Oecologia 134(4): 505–510.

Lowry LF, Frost K (1984) Food and feeding of bowhead whales in western and northern Alaska. Scientific Reports of the Whales Research Institute 35:1–16.

MacAvoy SE, Bacalan V, Kazantseva M, Rhodes J, Kim K (2015) Sulfur isotopes show importance of freshwater primary production for Florida manatees. Mar Mamm Sci 31(2): 720–725.

Marcoux M, McMeans BC, Fisk AT, Ferguson SH (2012) Composition and temporal variation in the diet of beluga whales, derived from stable isotopes. Mar Ecol Prog Ser 471: 283-291.

Marini L, Moretti R, Accornero M (2011) Sulfur Isotopes in Magmatic-Hydrothermal Systems, Melts, and Magmas. Rev Mineral Geochem 73: 423–492.

Matthews CJD, Ferguson SH (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.

McCutchan JHJr, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102: 378–390

McMahon KW, Hamady LL, Thorrold SR (2013) A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol Oceanogr 58(2): 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(1): 69-76.

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mamm Sci 26(3): 509–572.

Niño-Torres CA, Gallo-Reynoso JP, Galván-Magaña F, Ecobar-Briones E, Macko SA (2006) Isotopic analysis of δ13C, δ15N, and δ34S “a feeding tale” in teeth of the longbeaked common dolphin, *Delphinus capensis*. Mar Mamm Sci 22(4): 831–846.

Peterson BJ, Fry B (1987) Stable Isotopes in Ecosystem Studies. Annu Rev Ecol Syst 18: 293–320.

Polischuk SC, Hobson KA, Ramsay MA (2001) Use of stable-carbon and-nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Can J Zool 79(3): 499-511.

Pomerleau C, Matthews CJD, Gobeil C, Stern GA, Ferguson SH, Macdonald RW, (2018) Mercury and stable isotope cycles in baleen plates are consistent with year-round feeding in two bowhead whale (*Balaena mysticetus*) populations. Polar Biol 41: 1881–1893. https://doi.org/10.1007/s00300-018-2329-y

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(5): 258–266.

Rees CE, Jenkins WJ, Monster J (1978) The sulphur isotopic composition. Geochim Cosmochim Acta 42(65): 377-381.

Reiss L, Häussermann V, Mayr C (2020) Stable isotope records of sei whale baleens from Chilean Patagonia as archives for feeding and migration behavior. Ecol Evol 10: 808 – 818.

Richards MP, Fuller BT, Sponheimer M, Robinson T, Ayliffe L (2003) Sulphur isotopes in palaeodietary studies: a review and results from a controlled feeding experiment. Int. J. Osteoarchaeol 13: 37 – 45.

Riekenberg PM, Camalich J, Svensson E, IJsseldijk LL, Brasseur SM, Witbaard R, Leopold MF, Rebolledo EB, Middelburg JJ, van der Meer MT, Sinninghe Damsté JS. (2021) Reconstructing the diet, trophic level and migration pattern of mysticete whales based on baleen isotopic composition. R Soc open sci 8(12): 210949.

Rita D, Borrell A, Víkingsson G, Aguilar A (2019) Histological structure of baleen plates and its relevance to sampling for stable isotope studies. Mamm Biol 99: 63–70

Stipanuk MH, Coloso RM, Garcia RAG, Banks MF (1992) Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr 122: 420−427

Storch KJ, Wagner DA, Burke JF, Young VR (1988) Quantitative study in vivo of methionine cycle in humans using [methyl-2H3]- and [1-13C]methionine. Am J Physiol 255: E322−E331

Storch KJ, Wagner DA, Burke JF, Young VR (1990) [1-13C; methyl-2H3]methionine kinetics in humans: methionine conservation and cystine sparing. Am J Physiol 258: E790−E798

Strauss H (2004) 4 Ga of seawater evolution: Evidence from the sulfur isotopic composition of sulfate. in Amend JP. Edwards KJ. Lyons TW. (eds). Sulfur biogeochemistry—Past and present: Spec Pap Geol Soc Am 379: 195–205

Tanz N, Schmidt H-L (2010) δ34S-Value Measurements in Food Origin Assignments and Sulfur Isotope Fractionations in Plants and Animals. J Agric Food Chem 58(5): 3139 – 3146.

Víkingsson GA, Pike DG, Desportes G, Øien N, Gunnlaugsson T, Bloch D (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.

Wang B, Yang W, McKittrick J, Meyers MA (2016) Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. Prog Mater Sci 76: 229–318.

Webb EC, Newton J, Lewis J, Stewart A, Miller B, Tarlton JF, Evershed RP (2017) Sulphur-isotope compositions of pig tissues from a controlled feeding study. STAR: Sci Technol Archaeol Res 3(1): 71 – 79.

Witteveen BH, Wynne KM (2016) Trophic niche partitioning and diet composition of sympatric fin (*Balaenoptera physalus*) and humpback whales (Megaptera novaeangliae) in the Gulf of Alaska revealed through stable isotope analysis. Mar Mamm Sci 32(4), 1319-1339.

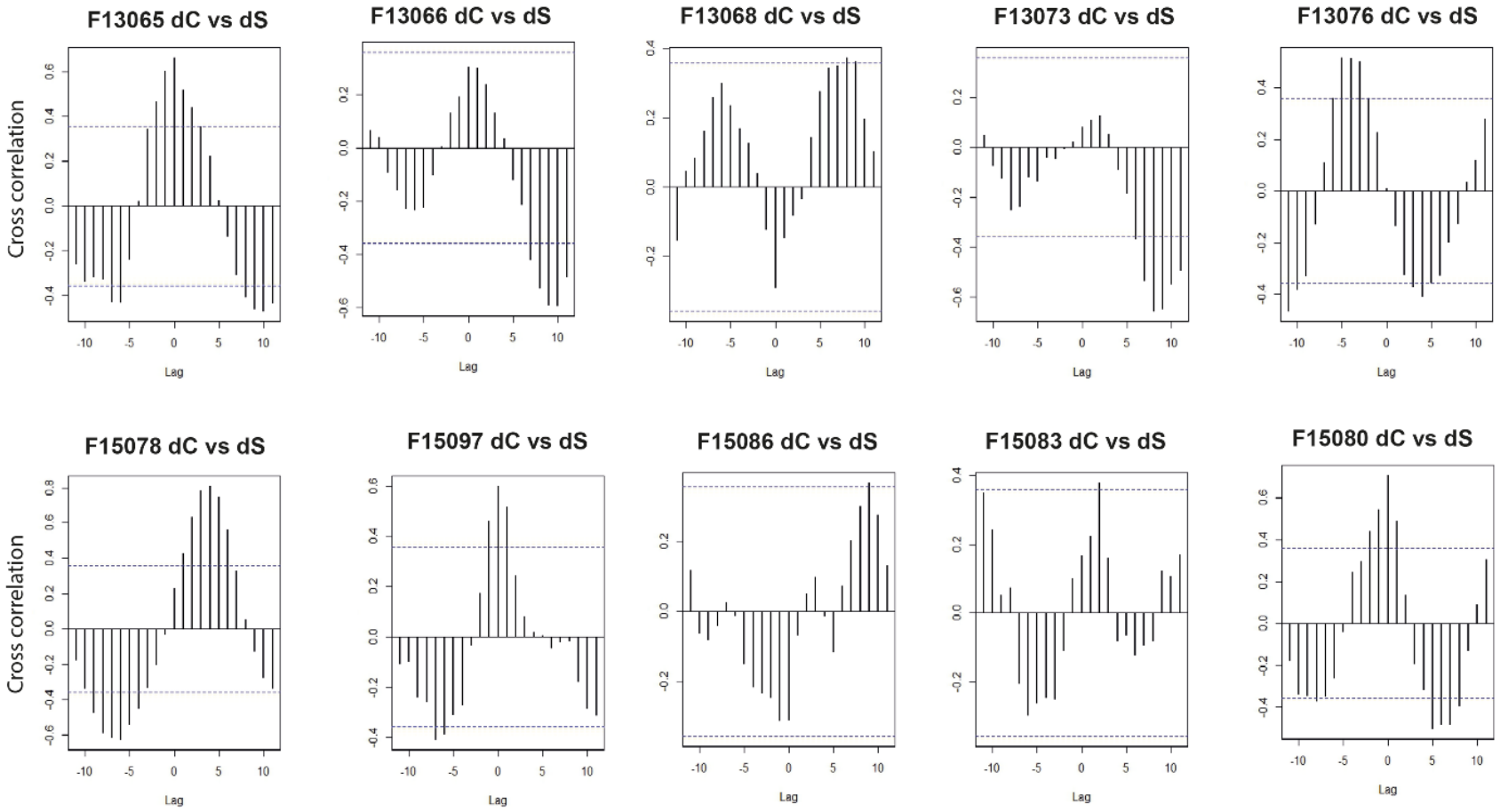


***Figure S1**: Results of the Cross-correlation functions, in which we compared the variation in the $\delta^{34}S$ and $\delta^{13}C$ values determined in the seriated subsampling points from the ten baleen plates analyzed. Dotted lines indicate the significance level.*

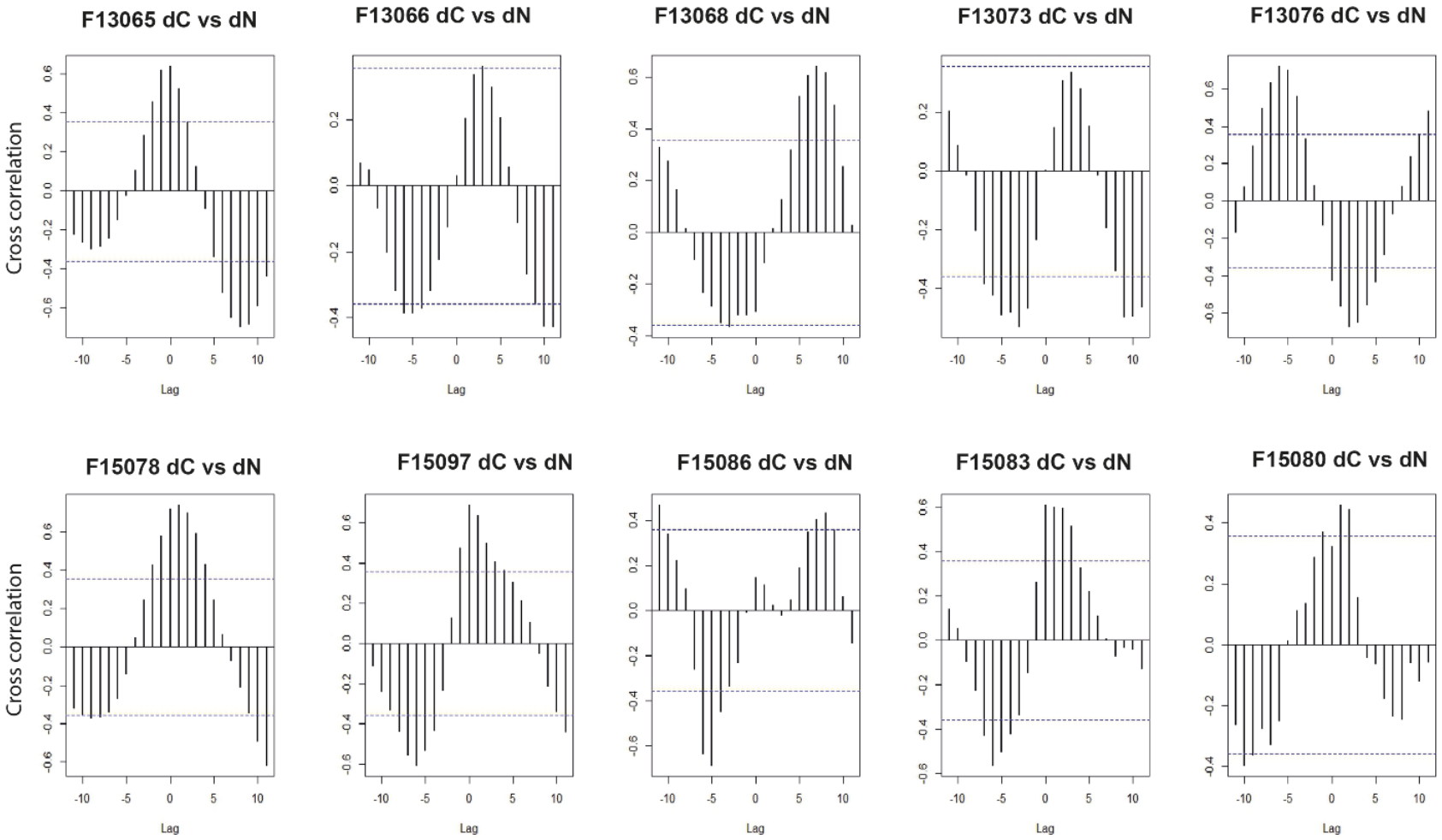
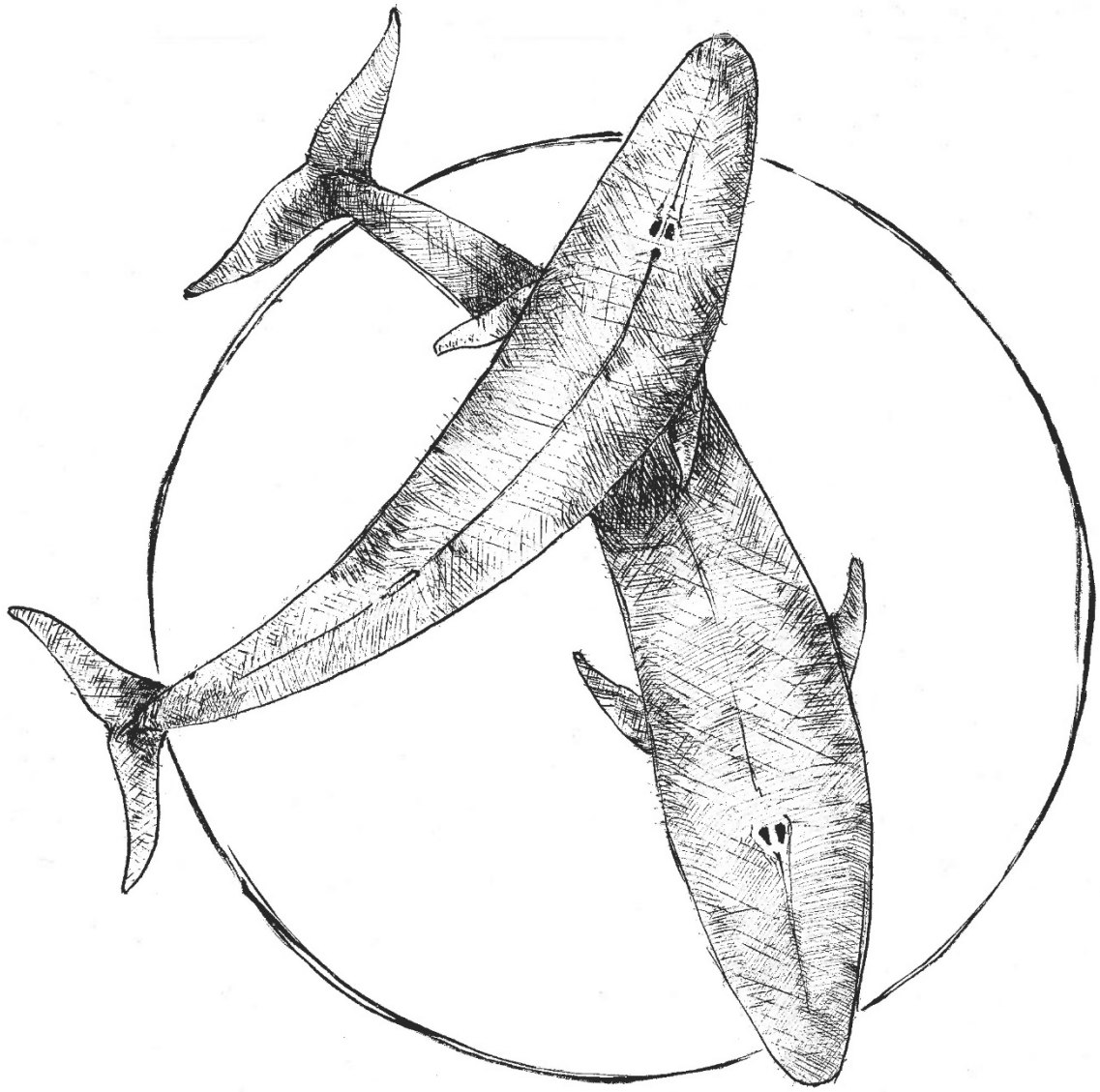


***Figure S2**: Results of the Cross-correlation functions, in which we compared the variation in the $\delta^{15}N$ and $\delta^{13}C$ values determined in the seriated subsampling points from the ten baleen plates analyzed. Dotted lines indicate the significance level.*

Table S1. *Parameters applied for the different GAM fitted for each baleen plate and stable isotope ratio. R square and deviance explained by the model are specified. Numbers in the outlier column represent the cm at which the sample was subtracted.*

Individual	Delta values	K	Outliers	Rsquare	Dev. Explained
F13065	δ34S	11		0.69	77.3
F13066	δ34S	Default		0.80	84.8
F13068	δ34S	Default		0.71	77.7
F13073	δ34S	Default	Cm 19	0.91	93.4
F13076	δ34S	10		0.77	82.9
F15078	δ34S	Default		0.85	88.4
F15097	δ34S	10	Cm 12, 15	0.59	71.2
F15086	δ34S	13		0.78	86.9
F15083	δ34S	Default		0.40	51.6
F15080	δ34S	Default		0.86	89.2
F13065	δ15N	Default		0.96	96.9
F13066	δ15N	Default		0.90	92.5
F13068	δ15N	Default		0.92	93.9
F13073	δ15N	Default		0.92	93.7
F13076	δ15N	Default		0.95	96.6
F15078	δ15N	13		0.90	93.3
F15097	δ15N	Default		0.90	92.5
F15086	δ15N	14		0.68	76.3
F15083	δ15N	Default		0.69	76.7
F15080	δ15N	11		0.77	84.3
F13065	δ13C	Default		0.95	96.4
F13066	δ13C	Default		0.94	95.3
F13068	δ13C	Default		0.88	90.5
F13073	δ13C	Default		0.90	92.9
F13076	δ13C	Default		0.86	89.7
F15078	δ13C	Default		0.97	97.8
F15097	δ13C	13		0.91	94.3
F15086	δ13C	Default		0.80	85.9
F15083	δ13C	Default		0.81	85.7
F15080	δ13C	Default	Cm 12	0.76	81.0



CAPÍTULO 3

Order within chaos: Genetically unrelated fin whales may migrate in dyads

Autores: **Raquel García-Vernet**[a], Diego Rita[a], Martine Berubé [bd], Julia Elgueta-Serra[a], Marina Pascual Guasch[a], Gísli Víkingsson[c], Asunción Borrell[a], Alex Aguilar[a]

[a] *Departamento de Biología Evolutiva, Ecología i Ciencias Ambientales, y IRBio, Facultad de Biología, Universidad de Barcelona*

[b] *Marine Evolution and Conservation, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands*

[c] *Marine and Freshwater Research Institute, Fornubúðum 5, 220 Hafnarfjörður, Iceland*

[d] *Center for Coastal Studies, 5 Holway Avenue, Provincetown, MA 02657, United States of America*

The life cycle of most baleen whales turns around annual migrations from low-latitude breeding grounds to high latitude feeding grounds. In most species, these migrations are traditionally considered to be carried out individually according to information acquired through vertical social learning during the first months of life. However, some recent studies have suggested a more complex scenario, particularly for the species of the *Balaenoptera* genus.

Here, we studied variation of δ15N values along the growth axis of the baleen plate from 24 fin whales to delve into their pattern of movements and to identify potential associations between individuals. The segment of baleen plate analyzed informed about two complete migratory cycles. We performed cluster analyses through two different methodologies and, whenever possible, we genotyped 20 microsatellite loci from DNA extracted from most individuals to determine potential existence of kinship. We detected a lack of significant population structure, which agrees with a winter dispersion strategy. However, and despite the overall large variability, several pairs of individuals with no kinship showed nearly identical isotopic patterns for two consecutive years, indicative of long-term association.

Although fin whales are often sighted travelling in pairs, this is the first evidence that such associations may be stable on the long-term. We also found some migratory patterns that, although not identical, were shared by different individuals. This led us to believe that some specific migratory regimes may be more beneficial than others and may also provide support to the hypothesis that some fin whales from the Icelandic feeding ground share the same migratory regime and destinations.

Order within chaos: Genetically unrelated fin whales may migrate in dyads

Raquel García-Vernet[1], Diego Rita[1], Martine Bérubé[2], Julia Elgueta-Serra[1], Marina Pascual Guasch[1], Gísli Víkingsson[3], Asunción Borrell[1], Alex Aguilar[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and IRBio, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain

[2] Marine Evolution and Conservation, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

[3]Marine and Freshwater Research Institute, PO Box 1390, Fornubúðum 5, 220, Hafnarfjörður, Iceland

[4] Center for Coastal Studies, 5 Holway Avenue, Provincetown, MA 02657, United States of America

*Correspondence: Raquel García-Vernet (Telephone number: + 34 93 402 1453; Barcelona, Spain).

Email: rakel.8592@gmail.com

1. ABSTRACT

The life cycle of most baleen whales turns around annual migrations from low-latitude breeding grounds to high latitude feeding grounds. In most species, these migrations are traditionally considered to be carried out individually according to information acquired through vertical social learning during the first months of life. However, some recent studies have suggested a more complex scenario, particularly for the species of the *Balaenoptera* genus. Here, we studied variation of $\delta^{15}N$ values along the growth axis of the baleen plate from 24 fin whales to delve into their pattern of movements and to identify potential associations between individuals. The segment of baleen plate analyzed informed about two complete migratory cycles. We performed cluster analyses through two different methodologies and, whenever possible, we genotyped 20 microsatellite loci from DNA extracted from most individuals to determine potential existence of kinship. We detected a lack of significant population structure, which agrees with a winter dispersion strategy. However, and despite the overall large variability, several pairs of individuals with no kinship showed nearly identical isotopic patterns for two consecutive years, indicative of long-term association. Although fin whales are often sighted travelling in pairs, this is the first evidence that such associations may be stable on the long-term. We also found some migratory patterns that, although not identical,

were shared by different individuals. This led us to believe that some specific migratory regimes may be more beneficial than others and may also provide support to the hypothesis that some fin whales from the Icelandic feeding ground share the same migratory regime and destinations.

2. INTRODUCTION

In ecology, migration is defined as the seasonal movement between regions that individuals or populations carry out to obtain more favorable conditions (Dingle and Drake, 2007). Many taxa perform migrations and, in some species, individuals migrate in groups, influencing each other and potentially leading to new social migratory behaviors (Shellard and Mallor, 2020). In comparison to other aspects of migration, such as departure time or migratory destinations, social factors have received limited attention (Couzin, 2018). This is particularly true in highly mobile oceanic species, as is the case with most baleen whales.

Most baleen whales undertake seasonal migrations with strategies that vary depending on the species and population (Stern and Friedlaender, 2018). Historically, baleen whale migrations have been considered as individual movements, alternating high-latitude feeding grounds with low-latitude breeding grounds. However, recent research has shown that the picture is more complex than previously thought, especially in the Balaenopteridae family (see for example Geijer et al. 2016, Silva et al. 2013). One of the most intriguing aspects is the effect of social interactions. Vertical culture, which is the transmission of cultural traditions from parents to offspring, is perhaps one of the clearest examples of this and appears to determine both migratory destinations and phenology (Clapham et al., 2008; Valenzuela et al., 2009; Whitehead and Rendell, 2015). It appears that calves learn these from their mothers during the first months of life. In some species, such as humpback whales (*Megaptera novaeangliae*) and southern right whales (*Eubalaena australis*), individuals have been found to show fidelity to the feeding and breeding grounds of their mothers, a fact that in the long-term end up shaping the structure and genetics of populations (Valenzuela et al. 2009, Baker et al. 2013, Carroll et al. 2015).

In mysticetes, research on social learning and cultural transmission between non-related individuals is scarce, and most studies have been focused on humpback whales, a species in which songs and feeding techniques have been demonstrated to be transmitted horizontally to non-related conspecifics (Garland et al. 2011; Allen et al. 2013). In addition, several studies have reported multi-year stable associations between

individuals in the feeding grounds (Weinrich 1991, Ramp et al. 2010, Ziegesar et al. 2021), although long-term relationships between individuals are considered uncommon. Knowledge of long-term social interactions in other baleen whale species, particularly in species from the *Balaenoptera* genus, is to our knowledge non-existent.

The fin whale (*Balaenoptera physalus*) is a cosmopolitan species and one of the most abundant baleen whales inhabiting the North Atlantic (Aguilar and García-Vernet, 2018). There is some uncertainty regarding stock structure within the North Atlantic but diverse scientific evidence suggest that fin whales from 2-4 breeding populations migrate to around 7 feeding areas during summer with considerable degree of site-fidelity to feeding areas (International Whaling Commission 2007, 2009, 2017; Víkingsson and Gunnlaugsson 2006). As it happens for the other *Balaenoptera* species, little is known about social interactions occurring between individuals. In general, the fin whale is considered non-gregarious, and the only known social bond is that between mother and calf (Aguilar and García-Vernet, 2018). However, fin whales are often seen swimming in pairs and, with less frequency, in larger groups (see for example Víkingsson et al. 2009), but the nature and duration of these associations are still unknown. Also, the obvious occurrence in a closed population of individuals that are genetically related to each other, such as parents/offspring, complete or half siblings, or even more loosely related individuals (e.g. Pampoulie et al., 2013), may facilitate or promote some degree of structuring in that population or of long-term association between the involved individuals.

Stable isotopes may provide an insight into these associations because their ratios in tissues reflect those from the environment in which the individual lives. They have been widely applied to investigate the migration patterns of a large number of species, including marine mammals (Hobson 1999, Newsome et al. 2010). From all the tissues in which stable isotopes can be determined, keratinous tissues, such as baleen plates, are particularly useful because their biochemical composition does not vary after the tissue is consolidated (Ramos and González-Solís 2012, Cherel et al. 2009). In addition, baleen plates experience a continuous growth, preserving a chronologically sequential record that reflects both the various environments visited by the whale and the changes that have occurred in its diet. Thanks to this, it has been possible to study variations in habitat use and diet during certain periods of the life cycle of individuals that would otherwise be impossible to monitor (Caraveo-Patiño et al. 2007, Matthews and Ferguson 2015, Eisenmann et al. 2016). Among the various elements that can be used for this purpose, the stable isotope ratios of nitrogen (from here $\delta^{15}N$ values) have received particular

attention because they integrate information from both the movements between regions with different baselines and the changes in diet (Kelly 2000, McMahon 2013).

Here, we studied variation of $\delta^{15}N$ values along the growth axis of baleen plates from different fin whales from the same feeding stock to better understand their pattern of movements, as well as to identify potential associations between individuals.

2. MATERIAL AND METHODS

2.1. Sampling

Baleen plates were obtained from 24 fin whales caught off south-western Iceland and flensed at the Hvalur H/F whaling station, Hvalfjörður, in 2013 (n = 5), 2015 (n = 9) and 2018 (n = 10). For all individuals except F18039 and the 5 individuals from 2013, skin samples were collected from the dorsal region of the body (n=18). Samples were transported from Iceland to Spain under CITES permit numbers, 15IS017MA, 18ISO36MA, ESBB00207/15I, ESBB00107/18I. After collection and during transport, baleen plates were preserved at -20ºC.

Once at the laboratory, the baleen plates were thawed. Any adhered materials were removed with steel wool, and their surface was cleaned using a chloroform-methanol solution (2:1). Previous studies showed that gum has different $\delta^{15}N$ values to those of the baleen plate surface (Rita et al. 2019), so the former was carefully separated from the plate surface with a cutter and removed. Once cleaned, baleen plates were stored dry until the analysis.

The samples of baleen plate tissue were extracted with a Dremel 300 series drill in 1 cm intervals, starting from the proximal end of the baleen (the most recent tissue) to the most distal (the oldest tissue). 40 sampling points were considered from each baleen plate, which corresponds, approximately, to a period of 2 years (Aguilar et al. 2014).

2.2. Stable isotope analysis

Samples (approximately 0.3 mg each) were weighed into tin capsules and combusted at 1000°C to be analyzed using a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Flash 1112 elemental analyzer; CE Elantech, Lakewood, NJ, USA), coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (both from ThermoFinnigan, Bremen, Germany).

International isotope secondary standards of known $^{15}N/^{14}N$ ratios in relation to the atmospheric nitrogen (air), namely ammonium sulfate (IAEA N1; $\delta^{15}N$ = +0.4‰ and IAEA N2; $\delta^{15}N$ = +20.3‰), potassium nitrate (USGS 34; $\delta^{15}N$= −1.7‰), L-glutamic acid (USGS 40; $\delta^{15}N$= −4.6‰) and caffeine (IAEA 600; $\delta^{15}N$ = 1.0‰), were used to calibrate the system and compensate for any analytical drift over time. The reference materials used for the analysis were selected to ensure that the range of the reference values spanned those of the samples. All analyses were conducted at the Centres Cientifics i Tecnològics of the University of Barcelona (CCiT-UB).

Stable isotopes ratios are expressed following the delta (δ) notation. The relative variations of stable isotope ratios are expressed as per mil (‰) deviations from the predefined international standards according to the equation:

$$\delta^{15}N = (R_{sample} / R_{standard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the heavy-to-light isotope ratios ($^{15}N/^{14}N$) in the sample and in the reference standard (i.e. air), respectively. Analytical precision for repeat measurements of the reference material, run in parallel with the skin samples, was 0.3‰ for $\delta^{15}N$ values.

From now on, samples of each year (2013, 2015 and 2018) will be treated as independent datasets. All statistical analysis was performed using R (4.1.0 version).

2.3. Time series cluster analysis

To detect association or similarities between individuals in their $\delta^{15}N$ variation along the baleen plate we performed time series cluster analysis for each dataset using two different methodologies: one based on Dynamic Time Warping (DTW) and one based on Cross Correlation Functions (CCF)

2.3.1. Dynamic Time Warping (DTW) cluster analysis

DTW is an algorithm designed to find an optimal alignment between two time-dependent sequences that may contain different time-steps (Müller, 2007). DTW applies a lag to each point of a series to find the best fit on the other series. Unlike the cross-correlation, DTW can apply a different lag to each point, thus accounting for changes in baleen plate growth rate and sampling errors. This technique has the advantage of providing similarity values based on the overall shape of the time-series (Aghabozorgi et al. 2015). Therefore, DTW is useful when comparing baleen plates records, especially in segments where the stable isotope ratios change fast, as is the case in fin whales (García-Vernet et al. 2018).

Cluster analyses were performed in R (R version 4.1.0) for each dataset (i.e. whales sampled in 2013, 2015 and 2018) with the dtwclust package (Sardá-Espinosa 2017). We used a Sakoe-Chiba window, the size of which was 10% of the length of the series (Sardà-Espinosa 2017). Other settings were established as follows: distance measure: dtw2; centroid (prototyping function): DBA; type (clustering method): hierarchical; agglomeration method for hierarchical clustering: complete; other parameters: default. We plotted the results as dendrograms using the dendextend package.

2.3.2. Cross Correlation Functions (CCF) cluster analysis

Instead of warping the time series for finding the best alignment, as the preceding DTW algorithms do, CFF calculates the lag in which the two time series are most correlated by displacing one with respect to the other. Taking into account the lag, it measures the correlation between the two time series. This methodology is more conservative with the original shape of the time series, but it is liable of being more affected by outliers than DTW analysis. To mitigate this effect, we used an adaptation of the Manhattan distance to calculate the similarity between the aligned time series.

We performed CCF to each possible pair of individuals inside each dataset, this is: 10 combinations for the 2013 dataset; 36 combinations for the 2015 dataset, and 45 combinations for the 2018 dataset. To avoid the comparison of tissue synthetized during different seasons, we fixed a maximum lag of ±3 centimeters. We stored the optimum lag for each pair of baleen plates.

Dissimilarity between each pair of individuals was calculated using an adaptation of Manhattan distance:

$$Dissimilarity\ (adapted\ Manhattan\ distance) = \frac{\sum_1^n |\delta^{15}N_{ik} - \delta^{15}N_{j(k+lag)}|}{n}$$

Where i and j are two individuals from the same dataset, k is the sample position, n is the total number of samples being compared, and lag means the optimum lag estimated for the "ij" pair before performing the data extraction.

We estimated the robustness of the results using a jackknife-like technique. 1% of the values in each dataset (4 values for 2019 and 2015 and 2 values for 2013) were randomly extracted and the dissimilarity index was again estimated for all the possible pairs. These dissimilarity measures were used to perform a second clustering analysis using the function hclust with the default parameters. This process was repeated 10.000 times for each dataset and stored all produced models as a list of "phylo" objects.

We used the ape package (Paradis and Schiliep 2019) to obtain a consensus dendrogram using the function “consensus”. The proportion for a clade to be represented in the consensus tree was fixed as 0.95 (p = 0.95). We used the function “prop.clade”, which counted the number of times that bipartitions were present in all the trees computed, as an approximation of the clades support. We plotted the results as dendrograms using the dendextend package.

2.3.3. Adaptation of Manhattan distance – visualization

Finally, we visualized the results of the adapted Manhattan distance, used as dissimilarity measure in the CFF clustering. We calculated the Dissimilarity for each pair of individuals of the original dataset without extracting any datapoint, and performed a cluster analysis using the function hclust with default parameters.

We plotted the cluster results, together with its dissimilarity measures, by using the function heatmap. We applied a row scaling to improve the visualization. Row scaling does not play a role in the clustering analysis but only serves to display the colors representing the dissimilarity measures between pairs. To finish, we printed a list with each individual and the corresponding pairs that showed lower dissimilarity values.

2.4. Visual exploration

We considered that two or more whales were probably associated when they met the following requirements:

i) Individuals were grouped together by the DTW cluster analysis.
ii) Individuals were grouped together by the CFF cluster analysis.
iii) Individuals presented a low dissimilarity measure in the CFF clustering when all datapoints are analyzed (Material and Methods 2.3.3.) compared with their other possible pairs (top 1 in case of the 2013 dataset, top 2 in case of the 2015 and 2018 dataset).

For all pairs or groups of whales that met these 3 requirements, we visually explored the similarities between these individuals by plotting their raw data.

2.5. Genetic analysis

2.5.1. Laboratory analyses

For individuals for whom skin samples were available, total-cell DNA was extracted from tissue samples either by standard phenol:chloroform extractions (Sambrook *et al.* 1989) or using the Qiagen DNeasy™ Blood and Tissue Kit (Qiagen Inc.) according to the manufacturer’s instructions. Extracted DNA was re-suspended in 1x TE buffer

(10mM Tris-HCl, 1mM EDTA, pH 8.0). The sex of each sample was determined from extracted DNA by molecular sex determination as described previously by Bérubé & Palsbøll (1996a) and Bérubé & Palsbøll (1996b)

The genotypes were determined at 20 microsatellite loci, using the following oligonucleotide primers: EV001, EV037 and EV094 (Valsecchi & Amos 1996), GATA028, GATA098, GATA417, and TAA023 (Palsbøll *et al.* 1997), GT011 (Bérubé *et al.* 1998), GT023, GT211, GT271, GT310, and GT575 (Bérubé *et al.* 2000), AC087 and CA234 (Bérubé *et al.* 2005), GATA25072, GATA43950, GATA5947654, GATA6063318, GATA91083 (Bérubé *et al.*, in prep). Samples were genotyped in multiplex PCR reactions, using the MM2X™ Multiplex kit Plus (Qiagen Inc.) in 5µL reaction volumes. The thermos-cycling conditions were: 2 min. at 94°C, followed by 35 cycles at 94°C (30 sec.), 57°C (90 sec.) and 72°C (30 sec.) followed by a final cycle at 68°C (10 min.). The PCR products were separated by capillary electrophoresis using an ABI 3730 (Applied Biosystems ABI Prism™ 3730). The size of the amplification products was estimated using the Genescan ROX-500 size standard (Applied Biosystems Inc.) and GENEMAPPER™ (v. 4.0; Applied Biosystems Inc.).

2.5.2. Relatedness analysis

Allele frequencies, observed and expected heterozygosity as well as the probability of identity for each locus was estimated using the software GIMLET v. 1.3.3 (Valiere, 2002).

Relatedness between individuals was identified using the maximum likelihood approach implemented in ML-RELATE (Kalinowski *et al.* 2006). This software can accommodate for the presence of null allele; which is calculated using the Hardy-Weinberg test for excess homozygotes. If null alleles are present, they are then specify and all following calculations use the maximum likelihood estimates of the frequency of the null alleles.

2.5.3. Relationship analysis

The relationship (i.e., unrelated, half-sibling, full-sibling, parent-offspring) between pairs of individuals was investigated using ML-relate. ML-relate indicates the relationship (R) with the highest likelihood LnL(R) and specifies how much lower the log-likelihood Delta Ln(L) are for the other relationships. The confidence was estimated with levels of confidence of 0.75, 0.50 and 0.25.

For the individuals with similar stable isotopes profiles, we then statistically assessed the reliability of our results by performing a “specific hypothesis test of relationship”, implemented in ML-relate, that attempted to exclude an alternative relationship (here

"Unrelated") by performing 50,000 simulations. We considered that the relationship was much more likely than "Unrelated" when *p*-values were lower than 0.05 (Kalinowski et al. 2006).

3. RESULTS

The total number of samples analyzed from the 24 fin whale baleen plates was 960. Mean and standard deviations of $\delta^{15}N$ values for each individual are detailed in Table S1.

3.1. Time series cluster analysis– DTW and CCF clustering

Results from the DTW and the CFF hierarchical clustering are shown in Figure 1. The topology of dendrograms differed between the two methodologies. CCF dendrograms presented polytomies, which corresponded to the clades with $p < 0.95$. Although we found large variability between individuals, several pairs or small groups of individuals were consistently grouped together, regardless of the clustering method.

Dendrograms for the 2013 dataset showed one common pair, composed by the individuals F13076 (male) and F13066 (male). The remaining dendrogram structure was different between the two methodologies (Figure 1A).

Dendrograms for the 2015 dataset differed in their general topology. However, two pairs were common between the two methodologies: F15080 – F15088 (male, male) and F15083 – F15079 (female, female). In CCF cluster analysis, only three dendrogram clades were well supported: those of the aforementioned pairs, and the cluster with individuals F15080, F15088, F15084 and F15086 (two males and two females), that were also grouped in the DTW cluster (Figure 1B).

Finally, dendrograms for the 2018 dataset also presented differences in the general topology. We only found one common pair in both dendrograms, composed by individuals F18054 (female) and F18043 (female). All the remaining tree topologies differed between methodologies, although we found a consistent group of three individuals (F18050, F18007, F18009, one female and two males) in them. In addition, CFF analyses detected two more pairs, although they were not supported by DTW analyses (Figure 1C).

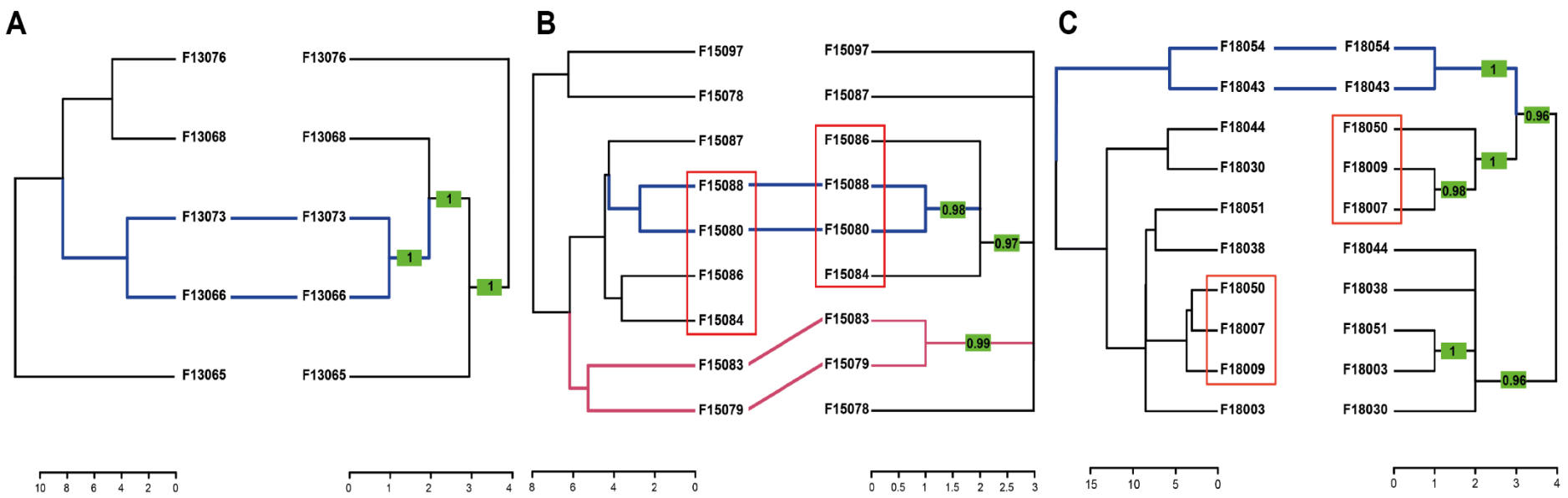


***Figure 1**: Dendrograms showing the results for 2013 (A), 2015 (B) and 2018 (C) datasets. Results from the DTW clustering are presented at the left dendrogram of each panel, and results from the CFF clustering are presented at the right. Colored lines show common branches and groups for both methodologies. Red squares show groups that are composed by the same individuals, although the internal structure is not maintained. Finally, in the CFF clustering, branches in which p>0.95 are indicated inside green boxes.*

3.2. Adaptation of Manhattan distance – visualization

To visualize the dissimilarity between each pair of individuals, we plotted our results using the heatmap function in R, and we printed a list with the individuals and the corresponding pairs that showed lower dissimilarity values, i.e. more similarity (Figure 2, Table S2).

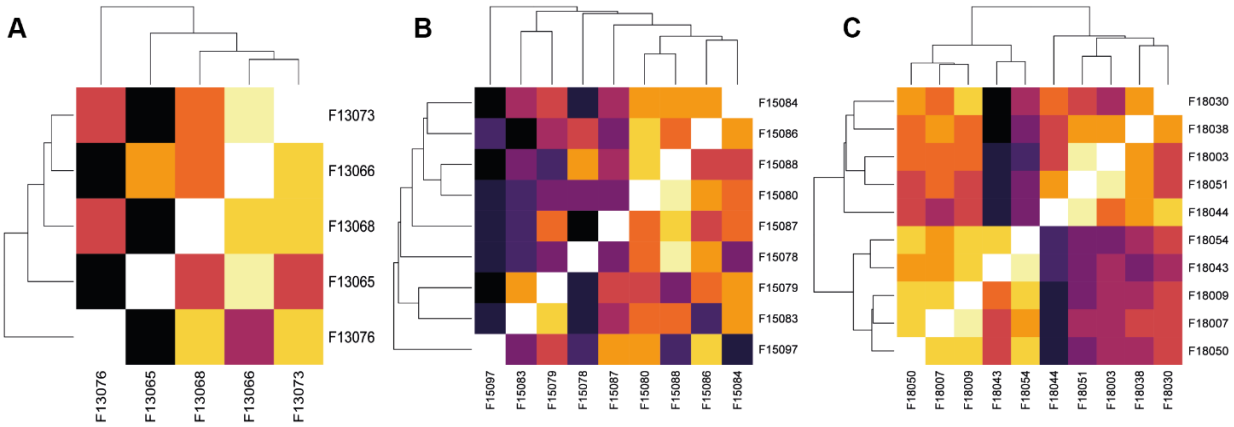


***Figure 2**: Heatmap showing the results of the CCF clustering and its dissimilarity values (based on the adapted version of the Manhattan distance), for 2013 (A), 2015 (B) and 2018 (C) datasets. CFF clusters were performed using all the data of each dataset, without extracting any point. Colors of the heatmap are indicative of the dissimilarity values between each pair of whales. Lighter colors indicate higher similarity between individuals.*

In general, we observed a great variability among individuals, without any clear group structure apart from the similarities between specific individuals. This result was in concordance with the low supports obtained in the CFF dendrograms topologies (Figure 1). The only exception was found in the 2018 dataset (which corresponds with the best supported dendrogram of the CFF clusters), where we found a group of whales with low dissimilarity measures among all the individuals. The group (showed in Fig 2 C; yellowish lower left quarter) was composed of whales F18050, F18007, F18009 (which were also grouped in the previous analyses) and F18043 and F18054 (which were grouped as a pair in the previous analyses).

3.4. Visual exploration

Four pairs of fin whales met all the similarity requirements. Their $\delta^{15}N$ patterns along the baleen length are shown in Figure 3. In addition, we plotted the $\delta^{15}N$ patterns of two more groups of whales that also clustered together (Figure S1, Figure S2), although the internal structure was not the same in the two methodologies.

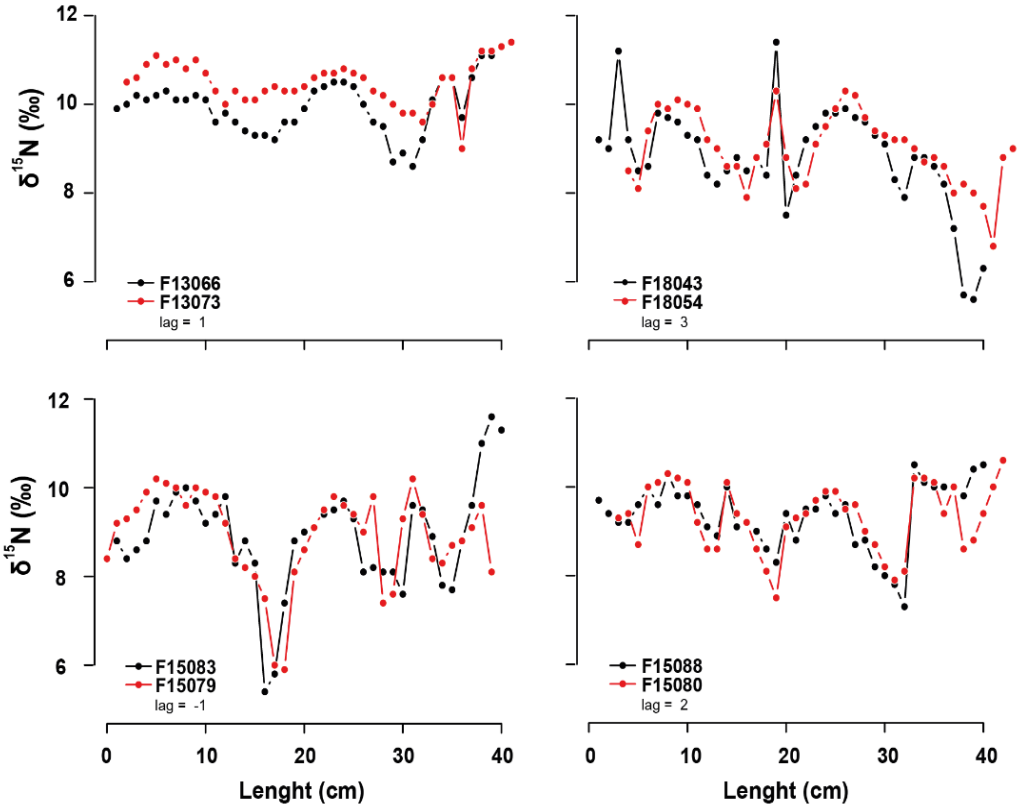


***Figure 3**: $\delta^{15}N$ patterns along the baleen length of the pairs of individuals which met all the similarity requirements: one pair from 2013 (F13076–F13066), two pairs from 2015 (F15080-F15088, F15083-F15079) and one pair from 2018 (F18043-F18054)*

Contrasting with the variability found in all the datasets, some pairs of individuals, detected through the clustering analyses, showed similar patterns along all the baleen plate growth axis, being in some cases (see for example F15088 and F15080) practically identical (Figure 3). In addition, one group of three (F18050, F18007, F18009) and another group of four whales (F15080, F15088, F15084 and F15086) also showed similar patterns (Figure S1), particularly between individuals F15080, F15088 and F15084.

3.5. Genetic Analyses

DNA was extracted from 18 individuals, 9 samples from 2015 and 9 samples from 2018. Standard statistics of the microsatellite loci are summarized in Table S3. None of the microsatellite loci deviated from expected Hardy-Weinberg genotypic frequencies. The probability of identities were estimated between 1.19×10^{-2} and 2.91×10^{-1} while the sibling probability of identities range between 2.93×10^{-1} and 4.90×10^{-1}. Two microsatellite loci presented possible presence of null allele, GATA028 and GATA43950,

The relatedness values (R) between fin whales from 2015 and 2018 (Table S4) were estimated between 0 and 0.21. However, no relationships could be assessed with a significant level of confidence (0.05 level of significance). An additional specific test was performed for the individuals with similar stable isotopes profiles, to ensure that the individuals were not related. The p-value for all the comparisons was higher than 0.05, supporting the previous results.

We would like to note that with 20 microsatellite loci, the statistical power to identify half-siblings is much lower than to identify parent-offspring pairs. In our study, the resulting r values are very low (below 0.15, non-significant), therefore, we consider that the hypothesis that our individuals are unrelated is plausible.

4. DISCUSSION

Understanding baleen whale migratory behavior remains challenging, especially when individuals are outside the feeding grounds. With the aim of contributing to fill this gap of knowledge, we analyzed $\delta^{15}N$ values in every cm along the growth axis of 24 fin whale baleen plates. Assuming a mean growth rate of the fin whale baleen as 20cm (Aguilar et al., 2014), the total period embraced by the study corresponds to 2 years, that is, the two most recent complete migratory cycles.

In most baleen, $\delta^{15}N$ values showed oscillations along the baleen growth axis. However, the variability of these oscillations between individuals was very large, which explains why the different cluster methodologies failed to identify a common structure. Compared to other baleen whales whose migratory behavior is better known, such as humpback whales, the migration of the *Balaenoptera* species seems to be less regular and predictable, particularly in the North Atlantic, and individuals appear to disperse in offshore waters during winter (Mackintosh 1966, Payne and Webb 1971, Whitehead and Rendell, 2015; Aguilar and García-Vernet, 2018). Winter dispersal may provide some advantages, such as a better chance of feeding opportunistically during migrations and the breeding season (Silva et al. 2013, Silva et al. 2019), but it may also present some disadvantages, such as hampering communication and coordination between conspecifics.

Surprisingly, despite the overall large variability in $\delta^{15}N$ values oscillations observed among individuals, several of them, with no kinship, exhibited similar patterns along the growth axis of the baleen plate during the two consecutive years of isotopic record. This similarity was particularly noteworthy in four pairs, with some of them showing almost

identical patterns. We hypothesize that these results could denote the existence of a long-term relationship between the involved individuals.

The fin whale, as most other balaenopterids, is generally believed to be non-gregarious during winter (Aguilar and García-Vernet, 2018). Conversely, loose aggregations of up to tens of whales may be seen in the feeding grounds, although most commonly fin whales are encountered as singles or pairs (Víkingsson et al., 2009; Joiris et al. 2014); indeed, the larger aggregations are likely ephemeral and driven by the aggregation of prey. In some Balaenopterids, the smaller groups, usually of 2–3 individuals, seem to be more stable during the feeding season. For example, humpback whales show multi-year stable associations while they are at the feeding grounds, mostly involving pairs of individuals (Weinrich 1991, Ramp et al. 2010, Ziegesar et al. 2021). While in this species the main reason behind the association may be having a higher success in feeding through reciprocity or by-product mutualism through coordinated feeding behavior (Wiley et al., 2011), it has also been suggested that pairs involving one male and one female may be related to a breeding strategy (Weinrich 1991). However, it is considered improbable that long-term relationships are maintained throughout the year, because the associations in the feeding grounds are different than those in the breeding grounds (Ramp et al. 2010).

Other Balaenopterids, and very particularly fin whale, depart from this behavior. While at the feeding grounds, the fin whale does not often engage in cooperative feeding and its migratory and breeding behavior is apparently very different than that of humpback whales. Thus, the winter dispersion that appears to be the rule in fin whales evokes a scenario in which collective migration and long-term associations are unlikely. Nevertheless, in other groups of animals, collective migratory strategies may evolve even when social encounters are rare, demonstrating that social interactions still may play an important role in sparse organisms (Guttal and Couzin 2010).

Fin whales, and other baleen whales such as blue whales, produce low-frequency pulses which can propagate over long ranges in the ocean (Payne and Webb 1971). It seems that the main reason for producing these sounds is to attract females and indicate to them the location of prey patches (Croll et al. 2002, Romagosa et al. 2021). However, the sounds may also be used during migration and throughout the winter season to coordinate the behavior between individuals, as has been observed to happen in blue whales (Oestreich et al. 2020). Fin whales may be able to stay in acoustic contact over hundreds of kilometers, and can thus migrate as a group even when animals are spread out over a wide area and apparently travelling alone (Stern and Friedlaender 2018). The

sharp similitude between the δ15N value oscillations observed between the baleen plates of some individuals make it reasonable to hypothesize that individual fin whales may associate in a more stable manner than commonly considered.

Long-term associations may provide clear benefits to the individuals involved in such relationships. While group feeding is not so frequent and never as complex as in the humpback whale, cooperation and synchronic foraging yields a higher feeding success in fin whales (Canese et al. 2006, Ladrón de Guevara et al. 2008). This can be useful while at the feeding grounds, but might be even more important for obtaining opportunistic meals at the breeding grounds or during migrations, a situation in which prey is sparser and the energetics of feeding are more compromised. Also, travelling in small groups may also improve the detection of prey patches in low-productive areas by combining the sensory abilities of the various animals belonging to the same group (Couzin 2008, Stern and Friedlaender 2018).

Besides the above, associations between whales may also provide advantages to reproduction. For example, associations between males and females are frequent during the summer feeding season in blue whales (Sears and Perrin 2018, Schall et al. 2020) and in humpback whales (Weinrich 1991, Cartwright and Sullivan 2009), likely because males provide protection from harassment from other males to females and their calves (Cartwright and Sullivan 2009). In our study we did not find any male – female pair, but three out of four pairs were composed by females and alliances between females may also provide protection against male aggression (Cartwright and Sullivan 2009).

We did not find any specific genetic relationship between individuals that showed similar migratory patterns, so the associations cannot be linked to previously found matrilineal or other consanguinity ties within the collective of whales exploited off western Iceland (Pampoulie et al., 2013). Long-term associations between kin-related individuals are known to occur in odontocete species (see for example Rendell et al. 2019) and maybe in humpback whales, a species in which maternal lineages have an influence on social relationships (Weinrich et al. 2006). However, in this last species the associated individuals do not often show kinship (Valsechi et al. 2002, Pomilla and Rosenbaum 2006). It has been adduced that long-term association between non-related individuals could provide an opportunity to modify and improve the vertically learned migratory routes and then transfer them to offspring (Sasaki and Biro 2017, Berdahl et al. 2018). As a result of this, vertical and horizontal transference of migration, breeding and feeding grounds can create an collective memory (Stern and Friedlaender 2018). As has been shown in other animal groups, adopting the parental migratory route in adult life, rather

than dispersing randomly, may increase an individual's reproductive success because that strategy has already been proven to allow successful breeding (Harrison et al., 2010). Such behavior could have relevant consequences for the long-term stability of the population because, if all individuals with that knowledge are extirpated from the population, this information may be lost (Clapham et al. 2008).

Finally, we cannot discard the possibility that the similarities observed in the baleen $\delta^{15}N$ value oscillations may be due to factors other than stable associations of individuals. For example, some specific migratory regimes may be more beneficial and then be shared between individuals without the need of them being physically associated. This alternative explanation could be especially true for the groups detected during the clustering analyses. The group found in 2015 was composed of four individuals, while that found in 2018 was composed of three individuals. Since fin whales rarely travel in large groups (Víkingsson et al., 2009; Joiris et al. 2014), it seems unlikely that the observed grouping is the consequence of long-term spatial associations. Instead, the putative groups detected may reflect some degree of structure in the fin whales visiting western Icelandic waters in the summer. Thus, genetic analyses have shown that fin whales from the same feeding ground may use different breeding grounds (Bérubé et al. 1998), and this may be the case for western Iceland, where the community of whales visiting the region may be composed of a mixture of several breeding populations or sub-populations (International Whaling Commission, 2009). It may be the case that whales clustered together share the same migratory regime and destinations, and thus have a common and distinctive isotopic signal, without having necessarily to migrate spatially together. Alternatively, the grouping may reflect spatial and/or temporal segregation according to age and sex within the population, that has been demonstrated for fin whales in this area and elsewhere (Martin 1982, Bérubé et al. 2001), including the possibility of remaining in high northern latitudes even in the winter (Lydersen et al. 2020).

The stable isotope values alone cannot discriminate the different possible explanations here suggested, but other approaches and methodologies are required to resolve this issue. Clarification is likely to come with the combined use or satellite tracking (Silva et al., 2013; Jiménez López et al. 2019, Lydersen et al. 2020), advanced genetic analyses (Bérubé et al. 1998, Cabrera et al. 2019), the pattern of vocalizations (Delarue et al., 2009; Morano et al., 2012), or the use of other chemical and biochemical tracers such as fatty acids or indicative natural or xenobiotic markers (Hobbs et al., 2001; Olsen et al., 2003; Vighi et al., 2015; 2019).

In summary, despite all the variability in the δ15N value oscillations patterns observed between individuals, some of them present an almost identical migratory pattern along two consecutive years. This is taken as evidence of long-term associations between the involved individuals a finding that, to our knowledge, has not been previously reported for any *Balaenoptera* species. In addition, we found some groups of whales that showed similar –though not identical- patterns, which may reflect some degree of internal population structure.

7. ACKNOWLEDGMENTS

This study was part of project PID2020-119712GB-I00 funded by the Ministerio de Ciencia e Innovación of Spain, MCIN/AEI/10.13039/501100011033. R.G-V's work was funded through a Ph.D. APIF scholarship granted by the University of Barcelona and through the Spanish State Public Employment Service (SEPE). Authors are grateful to the staff of the MFRI who assisted in the fieldwork. The stable isotope analyses were conducted at the Centres Científics i Tecnològics (CCiT-UB) of the University of Barcelona. R.G-V wants to thank M. Ribalta for his valuable help with R.

6. REFERENCES

Aghabozorgi, S., Shirkhorshidi, A.S., Wah, T. Y. 2015. Time-series clustering – A decade review. Information Systems, 53: 16–38. DOI: 10.1016/j.is.2015.04.007.

Aguilar, A., García-Vernet, R. 2018. Fin whale, *Balaenoptera physalus*. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), Encyclopedia of Marine Mammals (Third Edition): 368-371

Aguilar, A., Giménez, J., Gómez-Campos, E., Cardona, L., Borrell, A. 2014. δ^{15}N value does not reflect fasting in mysticetes. PLoS ONE 9(3): e92288. DOI: 10.1371/journal.pone.0092288

Allen, J., Weinrich, M., Hoppitt, W., Rendell, L. 2013. Network-based diffusion analysis reveals cultural transmission of lobtail feeding in humpback whales. Science, 340: 485 – 488. DOI: 10.1126/science.1231976

Baker, C.S., Steel, D., Calambokidis, J., Falcone, E., González-Peral, U., Barlow, J., Burdin, A. M., Clapham, P.J., Ford, J.K.B., Gabriele, C.M., Mattila, D., Rojas-Bracho, L., Straley, J.M., Taylor, B.L., Urbán, J.,Wade, P.R., Weller, D., Witteveen, B.H., Yamaguchi, M. 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Marine Ecology Progress Series, 494: 291–306. DOI: 10.3354/meps10508

Berdahl, A.M., Kao, A.B., Flack, A., Westley, P.A., Codling, E.A., Couzin, I.D., Dell, A.I., Biro, D. 2018. Collective animal navigation and migratory culture: from theoretical models to empirical evidence. Philosophical Transactions of the Royal Society B: Biological Sciences, 373(1746), p.20170009. DOI: 10.1098/rstb.2017.0009

Bérubé, M., Palsbøll, P. 1996. Identification of sex in Cetaceans by multiplexing with three ZFX and ZFY specific primers. Molecular Ecology, 5(2): 283-287. DOI: 10.1046/j.1365-294X.1996.00072.x

Bérubé, M., Palsbøll, P. 1996. Erratum of identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. Molecular Ecology, 5 (4): 602, DOI: 10.1111/j.1365-294X.1996.tb00355.x

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo Di Sciara, G., Sears, R., Sigurjónsson, J., Urban-R, J., Palsbøll, P. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. Molecular Ecology, 7: 585–599.

Bérubé, M., Jørgensen, H., McEwing, R., Palsbøll, P.J. 2000. Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae.* Molecular Ecology, 9(12): 2181-2183. DOI: 10.1046/j.1365-294X.2000.105315.x

Bérubé, M., Berchok, C., and Sears, R. 2001. Observation of a male-biased sex ratio in the Gulf og St. Lawrence fin whales (Balaenoptera physalus): Temporal, geographical or group structure segregation? Marine Mammal Science, 17: 371–381. Blackwell Publishing Ltd Oxford, UK.

Bérubé, M., Rew, M., Skaug, H., Jørgensen, H., Robbins, J., Best, P., Palsbøll, P. 2005. Polymorphic microsatellite loci isolated from humpback whale, Megaptera novaeangliae and fin whale, *Balaenoptera physalus*. Conservation Genetics, 6(4): 31-636. DOI: 10.1007/s10592-005-9017-5

Cabrera A.A. et al. 2019. Fin whale (*Balaenoptera physalus*) mitogenomics: A cautionary tale of defining sub-species from mitochondrial sequence monophyly. Molecular Phylogenetics and Evolution, 135: 86-97. DOI: 10.1016/j.ympev.2019.02.003

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

Caraveo-Patiño, J., Hobson, K.A., Soto, L.A. 2007. Feeding ecology of gray whales inferred from stable-carbon and nitrogen isotopic analysis of baleen plates. Hydrobiologia, 586: 17–25. DOI 10.1007/s10750-006-0477-5

Carroll, E.L., Baker, C. S., Watson, M., Alderman, R., Bannister, J., Gaggiotti, O.E., Gröcke, D.R., Patenaude, N., Harcourt, R. 2015. Cultural traditions across a migratory network shape the genetic structure of southern right whales around Australia and New Zealand. Scientific Reports, 5: 16182. DOI: 10.1038/srep16182

Cartwright, R., Sullivan, M. 2009. Associations with multiple male groups increase the energy expenditure of humpback whale (*Megaptera novaeangliae*) female and calf pairs on the breeding grounds. Behaviour, 146: 1573-1600. DOI: 10.1163/156853909X458377

Cherel, Y., Kernaléguen, L., Richard, P., Guinet, C. 2009. Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. Biology Letters, 5: 830–832. DOI: 10.1098/rsbl.2009.0552

Clapham, P.J., Aguilar, A., Hatch, L.T. 2008. Determining spatial and temporal scales for management: lessons from whaling. Marine Mammal Science, 24(1): 183–201. DOI: 10.1111/j.1748-7692.2007.00175.x

Cote, J. et al. 2017. Behavioural synchronization of large-scale animal movements - disperse alone, but migrate together? Biological Reviews, Wiley, 92 (3): 1275 - 1296. DOI: 10.1111/brv.12279

Couzin, I.D. 2018. Collective animal migration. Current Biology, 28: R952–R1008. DOI: 10.1016/j.cub.2018.04.044

Craig, A.S., Herman, L.M., Pack A.A. 2002. Male mate choice and male–male competition coexist in the humpback whale (*Megaptera novaeangliae*). Canadian Journal of Zoology, 80: 745–755

Croll, D.A., Clark, C.W., Acevedo, A., Tershy, B., Flores, S., Gedamke, J., Urban, J. 2002. Only male fin whales sing loud songs. Nature, 417: 809-809. DOI: 10.1038/417809a

Delarue, J., Todd, S. K., Van Parijs, S. M., Di Iorio, L. 2009. Geographic variation in Northwest Atlantic fin whale (*Balaenoptera physalus*) song: Implications for stock structure assessment. The Journal of the Acoustical Society of America, 125(3), 1774-1782. DOI: 10.1121/1.3068454

Dingle, H., Drake, V.A. 2007. What is migration? Bioscience 57, 2: 113 – 121. DOI: 10.1641/B570206

Eisenmann, P., Fry, B., Holyoake, C., Coughran, D., Nicol, S., Nash, S.B. 2016. Isotopic evidence of a wide spectrum of feeding strategies in Southern Hemisphere humpback whale baleen records. PLoS ONE 11(5): e0156698. DOI: 10.1371/journal.pone.0156698

García-Vernet, R., Sant, P., Víkingsson, G., 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 Communications in Mass Spectrometry, 32: 1257–1262. DOI: 10.1002/rcm.8169

Garland, E.C., Goldizen, A.W., Rekdahl, M.L., Constantine, R., Garrigue, C., Hauser, N.D., Poole, M.M., Robbins, J., Noad, M.J. 2011. Dynamic horizontal cultural transmission of humpback whale song at the ocean basin scale. Current Biology, 21: 687–691. DOI: 10.1016/j.cub.2011.03.019

Geijer C.K.A., Notarbartolo Di Sciara, G., Panigada, S. 2016. Mysticete migration revisited: are Mediterranean fin whales an anomaly? Mammal Review, 46: 284–296

Guttal, V., Couzin, I.D. 2010. Social interactions, information use, and the evolution of collective migration. PNAS, 107 (37): 16172–16177. DOI: 10.1073/pnas.1006874107.

Harrison, X.A., Tregenza, T.O.M., Inger, R., Colhoun, K., Dawson, D.A., Gudmundsson, G.A., Bearhop, S. 2010. Cultural inheritance drives site fidelity and migratory connectivity in a long-distance migrant. Molecular Ecology, 19(24): 5484-5496.

Hobbs, K. E., Muir, D. C. G., Mitchell, E. 2001. Temporal and biogeographic comparisons of PCBs and persistent organochlorine pollutants in the blubber of fin whales from eastern Canada in 1971–1991. Environmental Pollution, 114(2), 243-254.

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

International Whaling Commission. 2007. Report of the Joint NAMMCO/IWC Scientific Workshop on the Catch History, Stock Structure and Abundance of North Atlantic Fin Whales, 23-26 March 2006, Reykjavík, Iceland. Journal of Cetacean Research and Management, 9 (SUPPL): 451–68.

International Whaling Commission. 2009. Report of the first intersessional RMP workshop on North Atlantic fin whales. Journal of Cetacean Research and Management, 11(SUPPL.), 425–452.

International Whaling Commission. 2017. Report of the Scientific Committee. Journal of Cetacean Research and Management, 18 (SUPPL): 86.

Jiménez López, M.E., Palacios, D.M., Jaramillo Legorreta, A., Urbán, J., Mate, B. R. 2019. Fin whale movements in the Gulf of California, Mexico, from satellite telemetry. PLoS ONE 14(1): e0209324. DOI: 10.1371/journal.pone.0209324

Joiris, C.R., Falck, E., D'Hert, D., Jungblut, S., Boos, K. 2014. An important late summer aggregation of fin whales *Balaenoptera physalus*, little auks *Alle alle* and Brünnich's guillemots *Uria lomvia* in the eastern Greenland Sea and Fram Strait: influence of hydrographic structures. Polar Biol, 37:1645–1657. DOI: 10.1007/s00300-014-1551-5

Kalinowski, S. T., Wagner, A. P., Taper, M. L. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes, 6(2): 576-579. DOI: 10.1111/j.1471-8286.2006.01256.x

Kao, A.B., Couzin, I.D. 2014 Decision accuracy in complex environments is often maximized by small group sizes. Proceedings of the Royal Society B, 281: 20133305. DOI: 10.1098/rspb.2013.3305

Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology, 78: 1–27.

Ladrón de Guevara, P., Lavaniegos, B.E., Heckel, G. 2008. Fin whales (*Balaenoptera physalus*) foraging on daytime surface swarms of the euphausiid *Nyctiphanes simplex* in Ballenas channel, gulf of California, Mexico. Journal of Mammalogy, 89(3):559–566. DOI: 10.1644/07-MAMM-A-067R2.1.

Lydersen, C., Vacquié-Garcia, J., 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. Scientific Reports, 10:16966. DOI: 10.1038/s41598-020-73996-z

Mackintosh, N.A. 1966. The distribution of southern blue and fin whales. In Whales, Dolphins, and Porpoises, Norris, K. S. Univ. of Calif. Press. Berkeley, Ed: 125-142.

Martin, A.R. 1982. Influence of date and position of capture on the length of fin whales taken by Iceland. Report of the International Whaling Commission, 32: 331–334.

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. Marine Ecology Progress Series, 522: 269–286. DOI: 10.3354/meps11145

McMahon, K.W., Hamady, L. L., Thorrold, S. R. 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnology Oceanography, 58(2): 697–714. DOI: 10.4319/lo.2013.58.2.0697

Morano, J. L., Salisbury, D. P., Rice, A. N., Conklin, K. L., Falk, K. L., Clark, C. W. 2012. Seasonal and geographical patterns of fin whale song in the western North Atlantic Ocean. The Journal of the Acoustical Society of America, 132(2), 1207-1212. http://dx.DOI.org/10.1121/1.4730890

Müller, M. 2007. Dynamic Time Warping. Information Retrieval for Music and Motion. 69 – 84. DOI: 10.1007/978-3-540-74048-3_4

Newsome, S.D., Clementz, M.T., Koch, P.L. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science, 26(3): 509–572. DOI: 10.1111/j.1748-7692.2009.00354.x

Oestreich, W.K., Fahlbusch, J.A., Cade, D.E., Calambokidis, J., Margolina, T., Joseph, J., Friedlaender, A.S., McKenna, M.F., Stimpert, A.K., Southall, B.L., Goldbogen, J.A. 2020. Animal-borne metrics enable acoustic detection of blue whale migration. Current Biology, 30(23): 4773-4779.DOI: 10.1016/j.cub.2020.08.105

Olsen, E., Grahl-Nielsen, O. 2003. Blubber fatty acids of minke whales: stratification, population identification and relation to diet. Marine Biology, 142(1), 13-24. DOI 10.1007/s00227-002-0934-2

Palsbøll, P.J., Bérubé, M., Larsen, A.H., Jørgensen, H. 1997. Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. Molecular Ecology, 6 (9): 893-895. DOI: 10.1046/j.1365-294X.1997.d01-214.x

Pampoulie, C., Benónísdóttir, S., Skaug, H.J., Elvarsson, B.Þ., Víkingsson, G.A. 2013. Genetic relatedness of North Atlantic fin whale Balaenoptera physalus in Icelandic waters. International Whaling Commission doc. SC/65a/RMP01, 1-7.

Paradis, E., Schliep, K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics, 35(3): 526–528.

Payne, R., Webb, D. 1971. Orientation by means of long range acoustic signaling in baleen whales. Annals of the New York Academy of Sciences, 188: 110–141. DOI: 10.1111/j.1749-6632.1971.tb13093.x

Pomilla, C., Rosenbaum, H.C. 2006. Estimates of relatedness in groups of humpback whales (*Megaptera novaeangliae*) on two wintering grounds of the Southern Hemisphere. Molecular Ecology, 15: 2541–2555. DOI: 10.1111/j.1365-294X.2006.02943.x

Ramos, R., González-Solís, J. 2012. Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. Frontiers in Ecology and the Environment, 10(5): 258–266 DOI:10.1890/110140

Ramp, C., Hagen, W., Palsbøll, P., Bérubé, M., Sears, R. 2010. Age-related multi-year associations in female humpback whales (*Megaptera novaeangliae*). Behavioral Ecology & Sociobiology, 64:1563–1576. DOI: 10.1007/s00265-010-0970-8.

Rendell L., Cantor M., Gero S., Whitehead H., Mann J. 2019 Causes and consequences of female centrality in cetacean societies. Philosophical Transactions of the Royal Society B, 374: 20180066. DOI: 10.1098/rstb.2018.0066

Rita, D., Borrell, A., Víkingsson, G., Aguilar, A. 2018. Histological structure of baleen plates and its relevance to sampling for stable isotope studies. Mammalian Biology, 99: 63 – 70. DOI: 10.1016/j.mambio.2019.10.004.

Romagosa M., Pérez-Jorge S., Cascão I., Mouriño H., Lehodey P., Pereira A., Marques T.A., Matias L., Silva M.A. 2021. Food talk: 40-Hz fin whale calls are associated with prey biomass. Proceedings of the Royal Society B, 288: 20211156. DOI: 10.1098/rspb.2021.1156

Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory press.

Sardá-Espinosa, A. (2017). Comparing time-series clustering algorithms in R using the dtwclust package. Vienna: R Development Core Team.

Sasaki, T., Biro, D. 2017. Cumulative culture can emerge from collective intelligence in animal groups. Nature, 8: 15049. DOI: 10.1038/ncomms15049

Schall E., Di Iorio L., Berchok C., Filún D., Bedriñana R., Buchan S.J., Opzeeland I.V., Sears R., Hucke-Gaete R. 2020. Visual and passive acoustic observations of blue whale trios from two distinct populations. Marine Mammal Science, 36(1): 365-374. DOI: 10.1111/mms.12643

Sears R., Perrin W.F. 2018. Blue whale. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals (Third edition): 110-114

Shellard, A., Mayor, R. 2020. Rules of collective migration: from the wildebeest to the neural crest. Philosophical Transactions of the Royal Society B, 375: 20190387. DOI: 10.1098/rstb.2019.0387

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(10): e76507. DOI:10.1371/journal.pone.0076507

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

Stern, S.J., Friedlaender, A.S. 2018. Migration and movement. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), Encyclopedia of Marine Mammals (Third Edition): 602 – 606.

Tyack, P., Whitehead, H. 1982. Male competition in large groups of wintering Humpback whales. Behaviour, 83 (1/2): 132-154.

Valenzuela, L.O., Sironi, M., Rowntree, V.J., Seger, J. 2009. Molecular Ecology. Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*). Molecular Ecology, 18: 782–791. DOI: 10.1111/j.1365-294X.2008.04069.x

Valiere, N. 2002. GIMLET: a computer program for analyzing genetic identification data. Molecular Ecology Notes, 2(3): 377-379. DOI: 1046/j.1471-8286.2002.00228.x

Valsecchi, E., Amos, W. 1996. Microsatellite markers for the study of cetacean populations Molecular Ecology, 5 (1): 151-156. DOI: 10.1111/j.1365-294X.1996.tb00301.x

Valsecchi, E., Hale, P., Corkeron, P., Amos, W. 2002. Social structure in migrating humpback whales (*Megaptera novaeangliae*). Molecular Ecology, 11: 507-518.

Vighi, M., Garcia-Nisa, I., Borrell, A., Aguilar, A. 2015. The fin whale, a marine top consumer, exposes strengths and weaknesses of the use of fluoride as ecological tracer. Chemosphere, 127, 229-237. DOI: 10.1016/j.chemosphere.2015.02.023

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?. Science of The Total Environment, 650, 1224-1230. DOI: 10.1016/j.scitotenv.2018.09.103

Víkingsson, G. A., and Gunnlaugsson, T. 2006. Stock structure of fin whales (Balaenoptera physalus) in the North Atlantic - Indications from non-genetic data. IWC/SC/M06/FW7.
https://www.researchgate.net/publication/267725313_Stock_structure_of_fin_whales_Balaenoptera_physalus_in_the_North_Atlantic_-_indications_from_non-genetic_data

Víkingsson, G.A., Pike, D.G., Desportes, G., Øien, N., Gunnlaugsson, T., Bloch, D. 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 Scientific Publications, 7: 49 – 72.

Weinrich, M. T. 1991. Stable social associations among humpback whales (*Megaptera novaeangliae*) in the southern Gulf of Maine. Canadian Journal of Zoology, 69: 3012-3018. DOI: 10.1139/z91-425.

Weinrich, M.T., Rosenbaum, H., Baker, C.S., Blackmer, A.L., Whitehead. H. 2006. The influence of maternal lineages on social affiliations among humpback whales (*Megaptera novaeangliae*) on their feeding grounds in the southern Gulf of Maine. Journal of Heredity, 97(3): 226–234. DOI: 10.1093/jhered/esj018.

Whitehead, H., Rendell, L. 2015. The cultural lives of whales and dolphins, Chicago: University of Chicago Press. DOI: 10.7208/9780226187426

Wiley, D., Ware, C., Bocconcelli, A., Cholewiak, D., Friedlaender, A., Thompson, M., & Weinrich, M. 2011. Underwater components of humpback whale bubble-net feeding behaviour. Behaviour, 148: 575-602.

von Ziegesar, O., Gill, S., Goodwin, B. 2021. Long-term associations and insights on social structure of the humpback whales in Prince William Sound, Alaska. Long-term associations and social structure of Humpback whales. bioRxiv preprint. DOI: 10.1101/2020.03.02.972828

***Table S1**: Mean and standard deviations of $\delta^{15}N$ values for each individual. Sex of each animal is also indicated.*

Individual	Sex	Year	Mean	SD
F13065	Female	2013	9.27	0.65
F13066	Male	2013	9.96	0.62
F13068	Female	2013	10.32	1.04
F13073	Male	2013	10.50	0.49
F13076	Male	2013	11.09	1.07
F15078	Male	2015	9.38	0.70
F15097	Female	2015	9.68	1.11
F15086	Female	2015	9.28	0.72
F15083	Female	2015	8.91	1.21
F15080	Male	2015	9.33	9.76
F15079	Female	2015	8.92	1.04
F15084	Female	2015	8.98	0.73
F15087	Male	2015	9.29	1.07
F15088	Male	2015	9.36	0.74
F18044	Female	2018	10.91	1.62
F18043	Female	2018	8.79	1.18
F18038	Female	2018	10.53	1.21
F18050	Female	2018	9.54	0.58
F18051	Female	2018	10.47	1.17
F18030	Female	2018	10.21	1.38
F18054	Female	2018	9.00	0.81
F18007	Male	2018	9.65	0.56
F18003	Male	2018	10.54	0.99
F18009	Male	2018	9.50	0.83

***Table S2**: Results from the adapted version of the Manhattan distance. For each individual, the corresponding pairs that showed lowest dissimilarity values are shown. This analysis was performed without extracting any datapoint from the dataset.*

Individual	Best Match	Second Best Match
F13065	F13066	F13073
F13066	F13073	F13065
F13068	F13066	F13073
F13073	F13066	F13068
F13076	F13073	F13068
F15078	F15088	F15086
F15097	F15086	F15087
F15086	F15080	F15084
F15083	F15079	F15084
F15080	F15088	F15086
F15079	F15084	F15083
F15084	F15080	F15086
F15087	F15088	F15080
F15088	F15080	F15078
F18044	F18051	F18030
F18043	F18054	F18009
F18038	F18007	F18030
F18050	F18009	F18007
F18051	F18003	F18044
F18030	F18009	F18050
F18054	F18043	F18050
F18007	F18009	F18050
F18003	F18051	F18038
F18009	F18007	F18050

Table S3. *Summary results for the 20 microsatellite loci genotyped in 18 individuals. Note. Na denote the number of alleles; N, the number of individuals typed; Ho and He, the observed and expected heterozygosity; PIth, probability of identity and Pisib, sibling probability of identity*

Locus	Na	Ho	He	PIth	PIsib
AC087	3	0,5	0,64	2,09E-01	4,84E-01
CA234	7	0,83	0,81	6,42E-02	3,63E-01
EV001	9	1	0,82	5,47E-02	3,53E-01
EV037	9	0,78	0,8	6,34E-02	3,67E-01
EV094	14	1	0,9	1,86E-02	3,05E-01
GATA028	16	0,83	0,92	1,19E-02	2,93E-01
GATA098	6	0,83	0,78	8,17E-02	3,78E-01
GATA417	12	0,94	0,88	2,43E-02	3,15E-01
GATA43950	7	0,5	0,61	1,80E-01	4,90E-01
GATA52422	11	0,72	0,83	4,87E-02	3,48E-01
GATA5947654	7	0,72	0,76	9,03E-02	3,91E-01
GATA6063318	9	0,67	0,64	1,57E-01	4,68E-01
GATA91083	6	0,72	0,71	1,29E-01	4,27E-01
GT011	7	0,89	0,85	4,30E-02	3,38E-01
GT023	8	0,72	0,78	8,01E-02	3,81E-01
GT211	7	0,83	0,78	7,78E-02	3,78E-01
GT271	6	0,61	0,68	1,57E-01	4,49E-01
GT310	6	0,83	0,64	1,75E-01	4,73E-01
GT575	7	0,72	0,75	9,00E-02	3,96E-01
TAA023	5	0,72	0,71	1,32E-01	4,28E-01

Table S4: *ML-Relate output matrix of maximum likelihood relatedness (R) between fin whales from 2015 and 2018. All the resulting r values are low (below 0.15, non-significant)*

	15078	**15079**	**15080**	**15083**	**15084**	**15086**	**15087**	**15088**	**15097**	**18003**	**18007**	**18009**	**18038**	**18043**	**14044**	**18050**	**18051**	**18054**
15078	1.000																	
15079	0.000	1.000																
15080	0.000	0.120	1.000															
15083	0.000	0.000	0.000	1.000														
15084	0.000	0.000	0.000	0.000	1.000													
15086	0.000	0.000	0.000	0.000	0.000	1.000												
15087	0.000	0.000	0.050	0.000	0.020	0.000	1.000											
15088	0.000	0.060	0.010	0.000	0.000	0.000	0.000	1.000										
15097	0.000	0.020	0.000	0.160	0.000	0.020	0.030	0.000	1.000									
18003	0.020	0.000	0.090	0.000	0.010	0.000	0.090	0.000	0.000	1.000								
18087	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.000	0.000	1.000							
18009	0.000	0.000	0.000	0.120	0.000	0.000	0.000	0.020	0.040	0.000	0.050	1.000						
18038	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.030	0.000	0.030	0.020	0.170	1.000					
18043	0.000	0.000	0.020	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	1.000				
18044	0.000	0.040	0.070	0.000	0.060	0.000	0.000	0.000	0.000	0.130	0.040	0.000	0.000	0.130	1.000			
18050	0.000	0.000	0.000	0.010	0.030	0.000	0.000	0.030	0.070	0.000	0.000	0.000	0.000	0.000	0.000	1.000		
18051	0.000	0.000	0.000	0.050	0.000	0.020	0.000	0.000	0.010	0.090	0.000	0.000	0.040	0.000	0.120	0.000	1.000	
18054	0.070	0.050	0.000	0.100	0.000	0.000	0.130	0.000	0.000	0.000	0.000	0.000	0.020	0.020	0.000	0.000	0.000	1.000

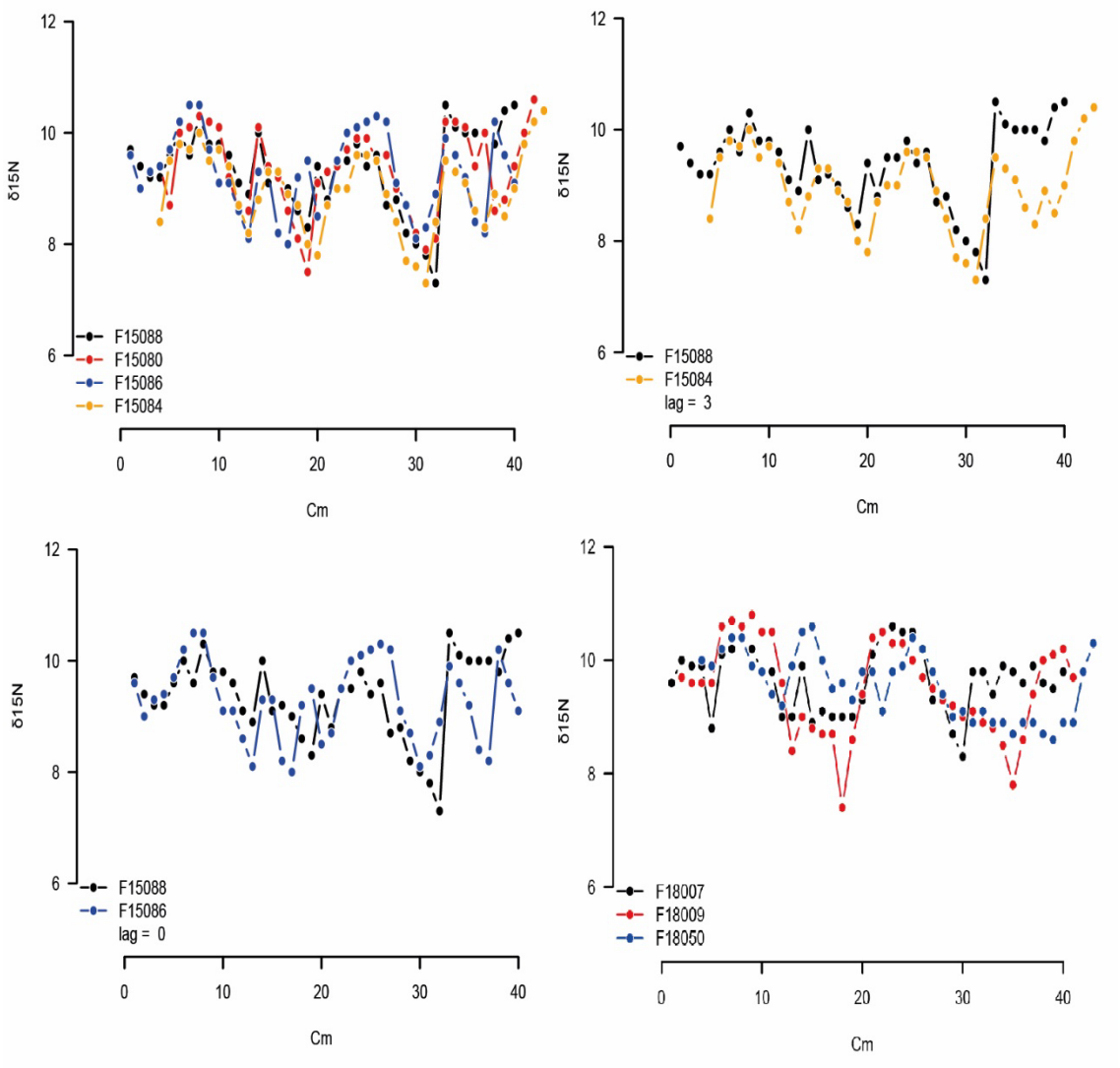
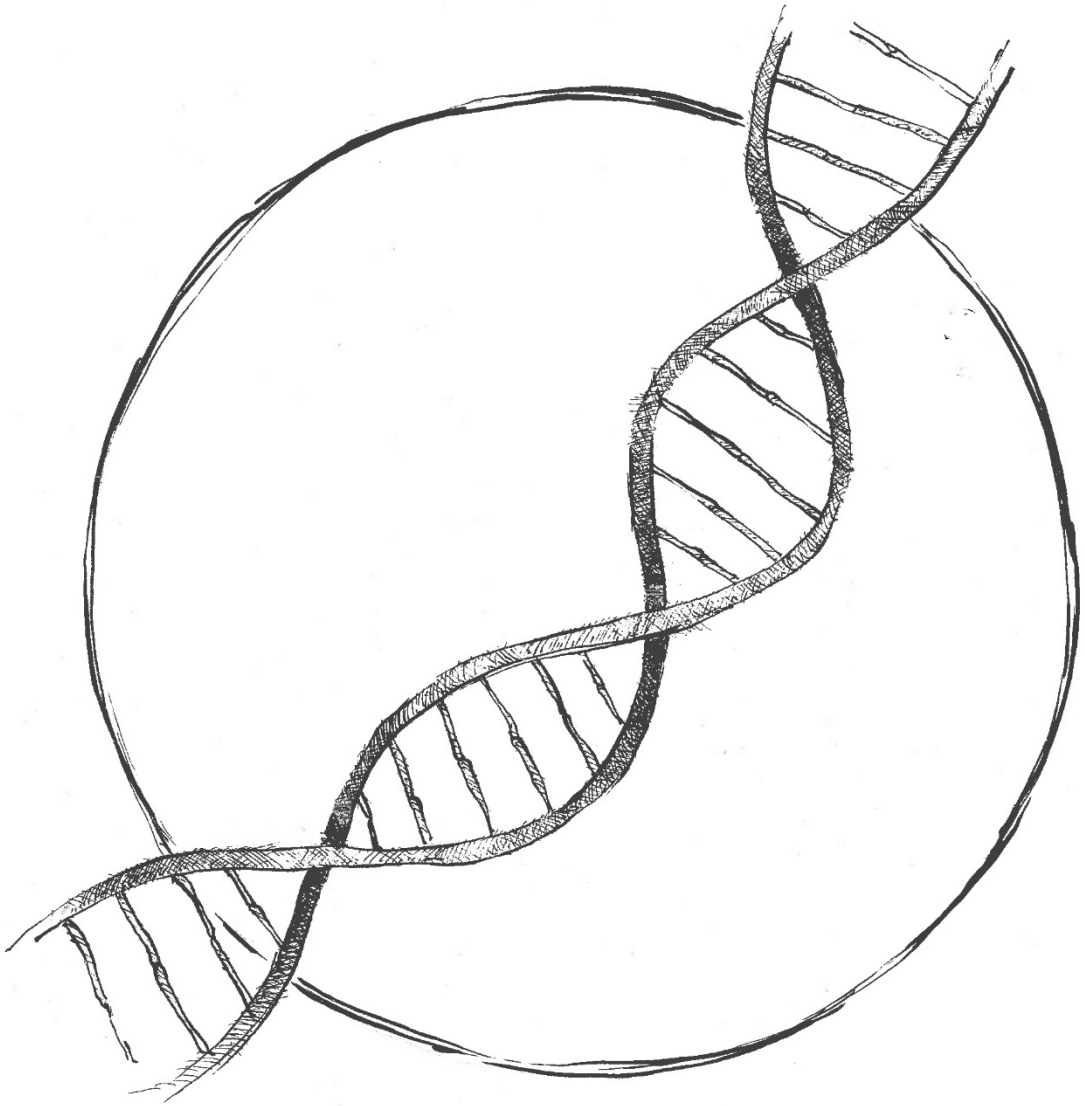


***Figure S1**: Consistent grouping of individuals from the 2015 and 2018 dataset. Panel on the top left shows the $\delta^{15}N$ patterns of individuals F15080, F15088, F15084 and F15086. F15080 and F15088 showed nearly identical values along the baleen plate growth axis. To facilitate the comparison, F15088 was plotted together with F15084 (top right) and F15086 (bottom left). We also detected a consistent grouping of individuals from the 2018 dataset: F18050, F18007, F18009 (bottom right)*



CAPÍTULO 4

CpG methylation frequency of TET2, GRIA2, and CDKN2A genes in the North Atlantic fin whale varies with age and between populations

Autores: **Raquel García-Vernet**[ab], Berta Martín[c], Miguel A. Peinado[c], Marta Riutort[b], Gísli Víkingsson[d], Alex Aguilar[a]

[a] *Departamento de Biología Evolutiva, Ecología i Ciencias Ambientales, y IRBio, Facultad de Biología, Universidad de Barcelona*

[b] *Departamento de Genética, Microbiología y Estadística, y IRBio, Facultad de Biología, Universidad de Barcelona*

[c] *Germans Trias i Pujol Research Institute (IGTP), Program of Predictive and Personalized Medicine of Cancer, Badalona, Spain*

[d] *Marine and Freshwater Research Institute, Fornubúðum 5, 220 Hafnarfjörður, Iceland*

Recovery rates for baleen whales that were decimated by exploitation vary between species and populations. Age determination is critical for the understanding of recovery trends and population structure, but determining age in free-ranging individuals remains challenging. Recent research has suggested that the methylation level of some genes in skin samples may provide age determinations with accuracy.

We selected nine CpG sites from three genes (TET2, CDKN2A, and GRIA2) and analyzed them in 40 skin samples from known-age individuals pertaining to two different populations of fin whales from the North Atlantic. We observed significant correlations with age in five CpG sites. We used three of these CpG sites to perform an epigenetic age estimation.

Predictions had a standard deviation of 2.94, but regression between observed and predicted ages showed a clear underestimation for older fin whales. For further development, we suggest: (1) screening for new CpG sites associated with age that exhibit higher variability between individuals, and (2) including older animals whenever the sampling allows it. We also observed subtle, but significant differences between the two populations studied in one of the CpG sites (TET2_CpG + 21). We attributed these differences to genetic differences or to the dissimilar environments that affect both populations.

Received: 12 May 2020 | Accepted: 22 February 2021

DOI: 10.1111/mms.12808

ARTICLE

Marine Mammal Science

CpG methylation frequency of *TET2*, *GRIA2*, and *CDKN2A* genes in the North Atlantic fin whale varies with age and between populations

Raquel García-Vernet[1,2] | **Berta Martín**[3] | **Miguel A. Peinado**[3] | **Gísli Víkingsson**[4] | **Marta Riutort**[2] | **Alex Aguilar**[1]

[1]Institute of Biodiversity Research (IRBio) and Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain

[2]Institute of Biodiversity Research (IRBio) and Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain

[3]Germans Trias i Pujol Research Institute (IGTP), Program of Predictive and Personalized Medicine of Cancer, Badalona, Spain

[4]Marine and Freshwater Research Institute, Reykjavík, Iceland

Correspondence
Raquel García-Vernet, Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Avinguda Diagonal 643, 08028 Barcelona, Spain.
Email: rgarciaver@ub.edu

Abstract

Recovery rates for baleen whales that were decimated by exploitation vary between species and populations. Age determination is critical for the understanding of recovery trends and population structure, but determining age in free-ranging individuals remains challenging. Recent research has suggested that the methylation level of some genes in skin samples may provide age determinations with accuracy. We selected nine CpG sites from three genes (*TET2*, *CDKN2A*, and *GRIA2*) and analyzed them in 40 skin samples from known-age individuals pertaining to two different populations of fin whales from the North Atlantic. We observed significant correlations with age in five CpG sites. We used three of these CpG sites to perform an epigenetic age estimation. Predictions had a standard deviation of 2.94, but regression between observed and predicted ages showed a clear underestimation for older fin whales. For further development, we suggest: (1) screening for new CpG sites associated with age that exhibit higher variability between individuals, and (2) including older animals whenever the sampling allows it. We also observed subtle, but

significant differences between the two populations studied in one of the CpG sites (TET2_CpG + 21). We attributed these differences to genetic differences or to the dissimilar environments that affect both populations.

KEYWORDS
cetaceans, epigenetics, marine mammals, molecular biology, population biology

1 | INTRODUCTION

Most baleen whale species have been subject to intense commercial exploitation for hundreds of years and particularly during the 20th century, when catches dramatically intensified (Clapham & Baker, 2018; Rocha et al., 2014). Whaling led to the depletion of many large whale populations worldwide until the International Whaling Commission (IWC) implemented management regulations that eventually led to the establishment of a moratorium on commercial whaling that started in 1985–1986 (Clapham & Baker, 2018). Since then, recovery of harvested populations has been unequal among regions and species and this highlighted the need for case-by-case monitoring and for a better understanding of population biology. In this context, substantial efforts have been invested in determining population structure and abundance, and in developing techniques that, in the absence of catches, might allow the determination of the main biological traits of individuals, such as sex, reproductive status, and age.

Age is critical to assess some basic population parameters such as somatic growth rate, physical maturation, age at onset of reproductive activity, longevity, or survival. In baleen whales, diverse techniques have been used to determine age. The one most widely applied, and considered to be the most reliable, has been the determination of the number of laminations or growth layer groups in the earplug, a structure located in the ear canal that is composed of successive depositions of wax and keratin that build up in layers over time. Early research demonstrated that one growth layer group is formed every year in most species (Lockyer, 1984). Counting of growth layer groups has been extensively used to obtain highly accurate age estimates in fin (*Balaenoptera physalus*) and sei whales (*Balaenoptera borealis*), but the technique has not worked well in other mysticete species such as blue (*Balaenoptera musculus*) or minke whales (*Balaenoptera acutorostrata*) (Lockyer, 1984). Other techniques, such as the degree of racemization of aspartic acid in eye globes (George et al., 1999; Olsen & Sunde, 2002; Yasunaga et al., 2017), the deposition of growth layers in the tympanic bullae (Christensen, 1995), or the occurrence of ridges in baleen plates (Chittleborough, 1959; Lubetkin et al., 2008) have also been attempted to age baleen whales but results have been uneven. In all cases the results obtained had little precision or, as it occurs with baleen plate ridges, the age record was limited to a short period of the whale's lifespan.

However, all these methods require samples from internal tissues that are impossible to obtain from living animals, so in recent years efforts have focused on developing techniques that permit age-determination through non-invasive means. Photo-identification studies have proven useful in species with easily recognizable morphological traits, such as humpback whales (*Megaptera novaeangliae*; Barlow & Clapham, 1997), but age determination of an individual is only possible when it is identified at birth and resighted subsequently. Consequently, application of this technique at the population level is only possible in studies with intensive research effort deployed for protracted periods of time. Moreover, it cannot be applied to species with poor fidelity to summer or winter grounds because resightings are sparse, or in those in which individual morphological identification is difficult, conditions that apply to most species of the *Balaenoptera* genus.

Biopsies collected from free-ranging individuals have since long become a ubiquitous technique used to monitor populations of cetaceans because they provide fresh skin samples with low impact on the sampled individuals

(Aguilar & Borrell, 1994; Aguilar & Nadal, 1984; Clapham & Mattila, 1993). A number of studies have attempted to use these samples to assess age. Telomere length in skin cells correlates with age in a number of species but does not appear accurate as an age predictor because telomere length at birth is highly variable between individuals and, afterwards, it is affected by environmental and physiological features (Dunshea et al., 2011; Olsen et al., 2014). The ratio between certain fatty acids present in the lipids of the blubber has also been found to be correlated with age in humpback whales (Herman et al., 2009), but the precision found in the age determination was low and, because lipids are to some degree dependent on the diet of whales, its application would require calibration for each population and may vary with time. More promising was the analysis of the methylation levels of selected genes, which in humpback whales appeared to provide high accuracy in age determinations (Polanowski et al., 2014; Riekkola et al., 2018). However, two of the three genes used in this study were later investigated in Antarctic minke whales and provided dissimilar results (Goto et al., 2020; Tanabe et al., 2020), thus suggesting differences between closely related species.

In vertebrates, methylation mainly occurs in the cytosines of cytosine-phosphate-guanine (CpG dinucleotides) and is associated with transcriptional repression of nearby genes (Bird & Wolffe, 1999; Deaton & Bird, 2011; Klose & Bird, 2006). Methylation is not static over time, but its rate changes during development (Smith & Meissner, 2013) and aging (D'Aquila et al., 2013). However, aging is not the only factor that exerts modifications in methylation patterns over time. Epigenetic modifications induced by the environment have been reported in many studies and, in some of them, these modifications have been associated with differences in gene expression (Feil & Fraga, 2012). Such environmentally induced changes might play a relevant role in adaptation processes, providing heterogeneity between and within populations, even if they are genetically homogenous (Flores et al., 2013). Therefore, it is not unreasonable to expect epigenetic differences, driven by the environment, between populations that have adapted to distinct conditions. Thus, the rate between methylation levels and age may not only be species-specific, but population-specific or even cohort-specific.

In this research we amplified and analyzed nine CpG sites of the three genes previously studied in humpback whales (Polanowski et al., 2014). *TET2* (ten-eleven translocation-2) is an evolutionarily conserved dioxygenase from the *TET* family. In humans, acquired *TET2* disruption and mutations have been related to hematopoietic malignancies (Mullinghan, 2009; Solary et al., 2014). *CDKN2A* (cyclin dependent kinase inhibitor 2A) encodes for several tumor suppressor proteins. Mutation and silencing of *CDKN2A* has been linked to several types of human cancers (Foulkes et al., 1997; Zhao et al., 2016). Finally, *GRIA2* (glutamate receptor Ia2/AMPA2) encodes for one of the four subunits that compose AMPA receptors, which are glutamate receptors. Hypermethylation of these three genes have been associated with aging in humans (Grönninger et al., 2010; Koch & Wagner, 2011).

The analyses were conducted on skin samples from fin whales from two separate populations (off western Iceland and northwestern Spain). Age of all individuals was determined through counts of growth layer groups in ear plugs that had been previously conducted for population biology assessments. The study had a double aim: (1) establishing whether the correlation between age and methylation levels observed in the three genes of the humpback whales previously studied were maintained in fin whales, and (2) examining interpopulation differences suggestive of environmental impact on the methylation rate of these CpG sites.

2 | MATERIALS AND METHODS

2.1 | Sample collection and age determination

The skin samples analyzed for the study were collected from fin whales caught by commercial whaling operations in two feeding grounds in the North Atlantic, which are managed as separate units by the International Whaling Commission (IWC, 2009). In the first one, located off the northwestern coast of Spain (from now on, referred to as the "Spanish population"), the samples were collected at the Caneliñas station from 20 individuals caught during the

1985 summer season. In the second, located off the western coast of Iceland (from now on, referred to as the "Icelandic population"), the samples were collected at the Hvalur H/F whaling station from 20 individuals caught during the summer of 1986. The skin samples were collected by A.A. and G.V. always from the central part of the dorsal region of the body to avoid epigenetic variation due to different sampling positions (Goto et al., 2020). Immediately after collection, samples were frozen at −20°C and preserved in this condition until analysis.

The age of individuals was determined by counting the growth layers present on a longitudinal section of their ear-plug core and following the methods described by Aguilar and Lockyer (1987). Each plug was examined by at least two researchers (A.A. and G.V. among others) and, in the case of obtaining different values in the multiple readings, the average of both estimates was used. The researchers that performed the ear plug readings have more than 30 years of experience. Individuals from the Icelandic population ranged between 7 and 27 years old, while individuals from the Spanish population ranged from 0 (two fetuses) to 49 years old.

2.2 | DNA extraction, bisulfite conversion, amplification, and pyrosequencing

DNA was isolated using a Speedtools Tissue DNA extraction kit (Biotools) and bisulfite converted using an EZ DNA methylation gold kit (Zymo Research, Irvine, CA). Both procedures were carried out following the corresponding manufacturer's instructions.

The genes *TET2*, *CDKN2A*, and *GRIA2* were amplified as in Polanowski et al. (2014). Since fin and humpback whales are closely related species (Árnason et al., 2018; Nikaido et al., 2006; Nishida et al., 2007; Sasaki et al., 2005), the primers designed by Polanowski et al. (2014) were used for the amplification of the three selected genes. PCR reactions were performed in a final volume of 30 μl, with 0.4 μl of Immolase DNA polymerase (Bioline 5 U/μl), 3 μl of 10× ImmoBuffer, 1.2 μl of 50 mM $MgCl_2$ solution, 0.4 μl of each primer at 0.25 μM and 6 μl of 0.5 mM dNTPs. When amplifying GRIA2, 6 μl of Betaine solution (Sigma) were added to the reaction to enhance the amplification. Thermocycling conditions for all three genes consisted of an initial step of 10 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 58°C, and 30 s at 72°C, with a final step of 5 min at 72°C.

All PCR products were visualized using gel electrophoresis. When weak amplification was detected in the agarose gel, samples were amplified again but the annealing temperature was set at 56°C instead of 58°C. For 31 samples, PCR reactions were performed twice. Of these, in seven samples PCR reactions were performed three times. Obtaining replicates of the PCR products allowed us to assess the potential error of the posterior pyrosequencing analysis.

PCR products were analyzed using a Pyromark Q96MD pyrosequencer (Qiagen GmbH, Hilden, Germany) at the PEBC platform (Bellvitge Biomedical Research Institute, IDIBELL) and the percentage of cytosine methylation at each CpG site was extracted using Pyro Q-CpG Software (Qiagen). To avoid confusion, the same notation as Polanowski et al. (2014) has been followed from now on and the position of the cytosine is indicated relative to the start codon of each gene. Negative or positive values indicate distance (5′ or 3′, respectively) in base pairs from the start codon.

2.3 | Statistical analysis

2.3.1 | Preliminary analyses

For each of the cytosine sites, presence of outliers was tested graphically using boxplots. To avoid a possible population effect, for each cytosine site the samples from Spain and Iceland were plotted separately. All the percentage values that fell outside the range of the whiskers were removed (that is, values located below or above the interquartile range × 1.5), but one exception was made when all PCR replicates of a same sample appeared as an outlier. In this case, we considered that it was a biological but not a methodological outlier and all values were maintained

(Figure S1). After removal of outliers, the means and standard deviation for each sample and CpG site were calculated. Data were tested for normality (Shapiro–Wilk test) and homoscedasticity (Bartlett test).

2.3.2 | Age effect

Relation between age and methylation was examined in depth for each individual position through a linear regression model (lm). In this case, models were fit considering the methylation percentage of each CpG position as the dependent variable and age as the covariate. Linear models were fitted for each population as well as for the whole set of samples. When model *p*-value was significant (<.05), the linear regressions were plotted and, in case of having PCR replicates, standard deviations were plotted as well at each individual point.

Samples with missing data were excluded from the following analyses. All CpG sites showing significant relationship with age were considered for being included into a multiple regression model. Multiple linear regressions were fitted only for combinations of CpG sites from different genes, to avoid multicollinearity issues between the independent variables (Polanowski et al., 2014).

Precision of multiple regression models was assessed through LOOCV. The LOOCV approach trains the model using all samples but one, which is excluded to test the model obtained. This procedure is repeated for every observation, and the final result is calculated by taking the mean of all individual calculations. RMSE (root mean square error) was used to determine the average model prediction error and to decide which of the ten combinations of two or three CpG sites was the best model.

2.3.3 | Population and sex effect

To assess the effect of other biological factors (sex and population) on methylation percentages, generalized linear models were fitted to the data. Each model was fitted considering each CpG site as the dependent variable, and sex and population were included as fixed factors with interaction. In addition, for *TET2_CpG + 21*, we reanalyzed the data through a Student's *t*-test to determine if differences between populations were still significant after extracting the samples of the Spanish population with ages that were not overlapping those of the Icelandic population.

Finally, to examine in depth the population effect on the methylation percentages on the CpG sites included in the best multiple regression model, we performed a principal component analysis (PCA). PCA was fitted only for samples without missing data.

Statistical analyses were carried out with the IBM SPSS 23 software package or in R 3.5.2. (R Core Team; http://www.R-project.org).

3 | RESULTS

3.1 | Determination of percentage of methylation

In total, nine CpG sites were successfully assayed through pyrosequencing: three for *TET2* (*TET2_CpG + 16*, *TET2_CpG + 21*, *TET2_CpG + 31*), three for *CDKN2A* (*CDKN2A_CpG + 297*, *CDKN2A_CpG + 303*, *CDKN2A_CpG + 309*), and three for *GRIA2* (*GRIA2_CpG + 202*, *GRIA2_CpG + 188*, *GRIA2_CpG + 183*). We were not able to obtain results for the fourth site of *TET2* (*TET2_CpG + 58*) or *CDKN2A* (*CDKN2A_CpG + 327*), but we obtained two additional sites in the *GRIA2* gene (*GRIA2_CpG + 188* and *GRIA2_CpG + 183*) that have been previously analyzed in minke whales (Tanabe et al., 2020).

TABLE 1 Total number of samples analyzed for each CpG site.

	Samples	Range of methylation	Difference between samples	Replicated samples	*SD* between replicas
TET2_CpG + 16	38	7.99–21.88	13.89%	26	1.68%
TET2_CpG + 21	38	0–15.66	15.66%	25	2.50%
TET2_CpG + 31	37	5.38–20.19	14.81%	24	1.48%
CDKN2A_CpG + 297	39	1.45–3.23	1.78%	30	0.43%
CDKN2A_CpG + 303	40	2.28–7.24	4.96%	31	0.67%
CDKN2A_CpG + 309	40	1.32–6.02	4.70%	28	0.69%
GRIA2 CpG + 202	36	0–5.79	5.79%	16	2.11%
GRIA2 CpG + 188	37	2.94–8.69	5.75%	9	2.01%
GRIA2 CpG + 183	36	1.05–5.89	4.84%	7	0.89%

Note: Range of methylation indicates the lowest and the highest values obtained for different samples in a given CpG site. Difference between samples indicates the difference between the highest and the lowest percentage of methylation for different samples in a given CpG site. Replicated samples column indicates the number of samples that were amplified and pyrosequenced for at least one additional time. *SD* between replicas indicates the mean standard deviation between replicates for a given CpG site.

We did not obtain results at each CpG site for all samples, getting results for all 40 samples only for the two last CpG sites of the gene *CDKN2A*. In the other cases, we obtained results from 36 to 39 samples, depending on the CpG site analyzed. Total range of variation between samples differed between genes and CpG sites. Differences between the highest and the lowest percentages of methylation were larger in *TET2* sites, followed by *GRIA2* and *CDKN2A* (Table 1).

For all CpG sites, we did not obtain results in all the replicates, so the number of replicates is variable depending on the gene and the CpG position analyzed (Table 1). All replicates were analyzed to calculate standard deviation (SD) and assess the reproducibility of our results. Average SD were variable between genes and CpG sites (Table 1). All CpG sites met the assumptions of normality and homoscedasticity, except *GRIA2_CpG + 183* site.

3.2 | Sources of variation in the percentage of methylation: Age

Linear regression models were fit for the whole data set and for each population separately at all CpG sites (Figure 1). When the two populations were analyzed together, linear models showed a significant effect of age on the methylation percentages in five CpG sites: three sites of *CDKN2A* ($p < .05$ for *CDKN2A_CpG + 297*, *CDKN2A_CpG + 303*, and *CDKN2A_CpG + 309*), the second CpG site in *TET2* ($p < .05$ for *TET2_CpG + 21*), and the second CpG site in *GRIA2* ($p < .01$ for *GRIA2_CpG + 188*). However, when each location was treated as a distinct data set, significant correlations were only found for the Spanish population in four out of five cases and for the Icelandic population in two out of five cases (Figure 1).

The five CpG sites with significant relationship with age were included in a multiple linear regression model. All possible combinations of two or three CpG sites were assessed with LOOCV and compared through their RMSE (root mean square error). For this comparison we chose RMSE as the indicator (and not MAE, the mean absolute error) because although both MAE and RMSE express average model prediction, RMSE gives higher weight to large errors, which are highly undesirable for age predictions. Five samples had missing data for some of the CpG sites and were excluded from the analyses, so multiple regressions were initially calibrated using 35 samples.

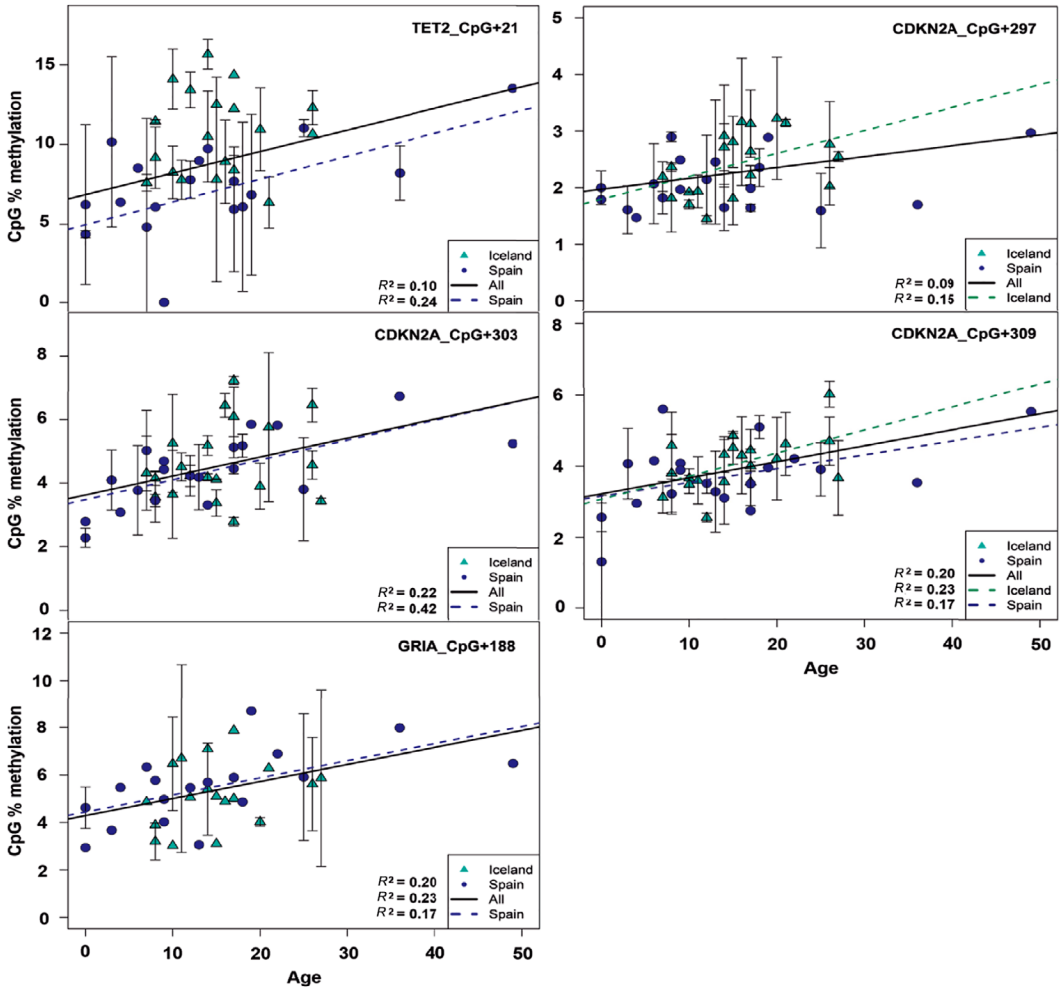


FIGURE 1 Linear regressions between age and % of cytosine methylation for each CpG site that showed significant correlation with age. Linear regressions were calibrated for each population and the whole data set (only those with correlations with $p < .05$ are shown). When data were available, standard deviations were plotted in their corresponding points. Adjusted R^2 is indicated for each regression line.

The multiple regression model with smallest RMSE had the combination of three CpG sites: *TET2_CpG + 21*, *CDKN2A_CpG + 303*, and *GRIA2_CpG + 188*. After checking the normality and homoscedasticity of the residuals, we removed an additional sample from the Spanish population that was placed as an outlier, so the model was run using the 34 remaining samples (Figure S2). The RMSE of the final model was 5.91 and the MAE was 4.87.

The precision of the multiple regression model was additionally assessed by checking the differences between the observed and predicted ages (residuals), that presented a standard deviation of 2.941 and a mean absolute difference of 4.264. The 95% prediction interval for the model was 11.84 (Figure 2).

Linear regression between known and predicted age is shown in Figure 2. The linear regression had a significant *y*-intercept of 6.603, indicating that our model initially overestimates young whale's age, but the slope of the regression showed that older whales' ages are highly underestimated. R^2 of the regression was 0.488, indicating that an important part of model's variation is due to other unknown factors.

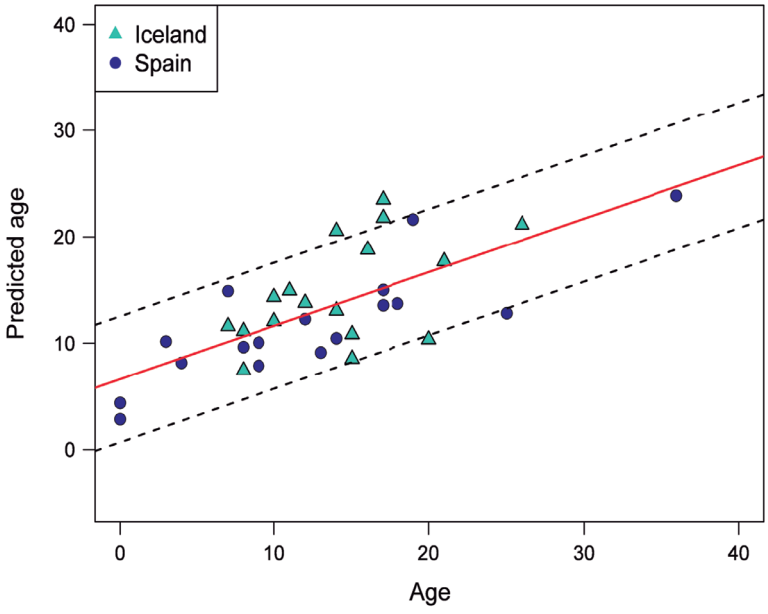


FIGURE 2 Regression between estimated and observed ages of the 34 samples included in the multiple regression model. Estimated ages are the results of the LOOCV analysis, which included *TET2_CpG + 21*, *CDKN2A_CpG + 303*, and *GRIA2_CpG + 188* sites. 95% prediction intervals are shown. The R^2 of the linear regression was 0.488, and the prediction intervals 11.84.

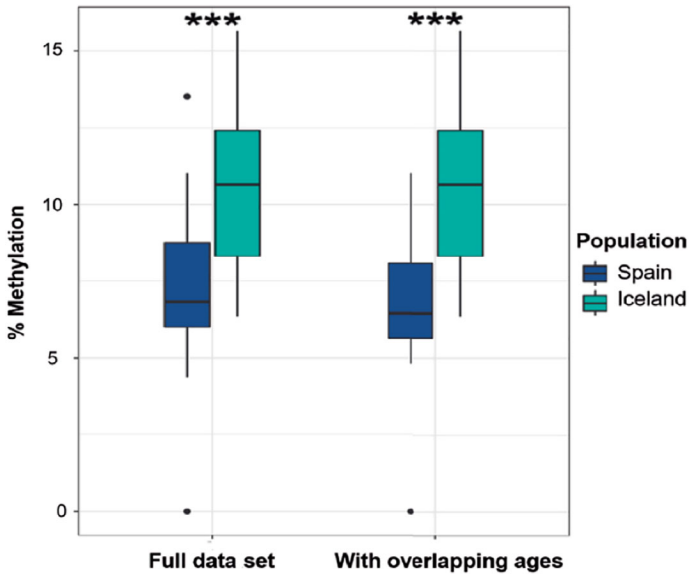


FIGURE 3 Boxplot distributions of the percentage of methylation in *TET2_CpG + 21* after the removal of outliers, using all individuals ("Full data set") or only those with overlapping ages in both populations ("With overlapping ages"). The top and bottom boundaries of each box indicate the 75th and 25th quartile values, respectively, and lines within each box represent the 50th quartile values.

3.3 | Sources of variation in the percentage of methylation: Populations

Generalized linear models showed no significant effect of sex in the methylation levels of any CpG site ($p > .05$), in accordance with previous results. However, a significant effect of population was detected in one CpG site (*TET2_CpG + 21*, $p < .001$). It should be noted that the age ranges of each population are not identical (7–27 years for Iceland population; 0–49 years for Spanish population), and in this CpG site a significant effect of age was detected ($p < .05$). Hence, all samples with ages that were outside the range of the Icelandic (7–27) were removed before reanalyzing possible differences between both populations through a Student's *t*-test. Significant differences were detected again between the Spanish and the Icelandic samples ($p < .001$; Figure 3), suggesting that both populations were exhibiting different methylation patterns in this CpG site.

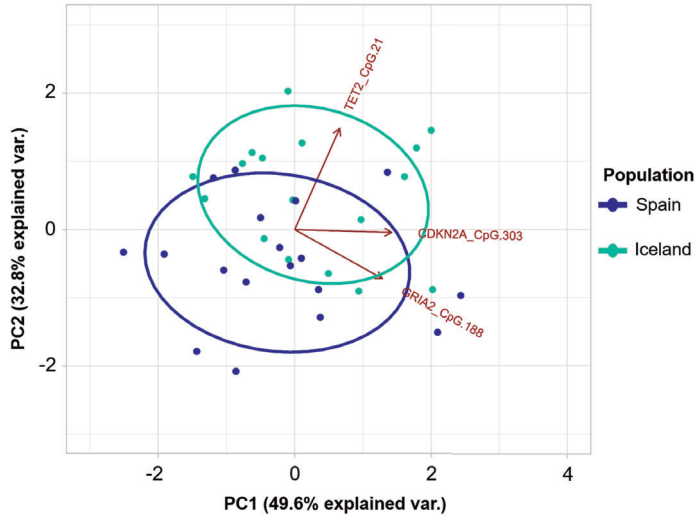


FIGURE 4 Results from the Principal Component Analysis (PCA). Each population is shown in different colors. For the first component (PC1), shown as *x*-axis, *CDKN2A_CpG + 303* and *GRIA2_CpG + 188* are the most important variables. For the second component (PC2), shown as *y*-axis, *TET2_CpG + 21* is the most important variable.

Finally, results from the PCA showed that the first two components explain most of the variation in the data, with a cumulative proportion of variance of 82.41%. For the first principal component (PC1), *CDKN2A_CpG + 303* contributed with 49.18% and *GRIA2_CpG + 188* contributed with 40.20%. For the second principal component (PC2), the most important variable was *TET2_CpG + 21*, which explained the 81.03% of the contribution. PC2 showed differences between the two populations, which were barely separated by PC1 (Figure 4).

4 | DISCUSSION

Although the exact mechanisms that drive the association between gene methylation and age are not totally understood, this correlation is well-established and several researchers have attempted to create protocols to infer human age through DNA methylation markers. Most of the efforts have focused on finding epigenetic markers that correlate with age in the forensically relevant tissues, such as blood (Bekaert et al., 2015; Hannum et al., 2013; Naue et al., 2017; Vidal-Bralo et al., 2016; Weidner et al., 2014), saliva (Bocklandt et al., 2011; Eipel et al., 2016), semen (Lee et al., 2015), and skin (Koch & Wagner, 2011). Outcomes of these studies were promising and provided high accuracy when predicting age, and this opened thrilling perspectives for the study and monitoring of other mammals, particularly those which live in the wild and are of restricted access.

In recent years, driven by the emergence of new cost-effective techniques, the use of epigenetic analysis in studies of ecology and evolution has experienced a substantial increase (De Paoli-Iseppi et al., 2017; Jarman et al., 2015; Verhoeven et al., 2016). Here we have analyzed CpG sites from three genes, previously applied with dissimilar results to infer age from skin samples of humpback and minke whales (Goto et al., 2020; Polanowski et al., 2014; Riekkola et al., 2018; Tanabe et al., 2020), to study their applicability to a different whale species.

Ranges of variation observed in our study between individuals' methylation levels for each CpG site were similar to those observed in previous studies (Goto et al., 2020; Polanowski et al., 2014; Tanabe et al., 2020). For the nine sites analyzed, we detected significant correlations with age in five, a finding that reflects the conserved relation between methylation patterns and aging in mammal species (Booth & Brunet, 2016; Sen et al., 2016). It is well known that methylation and aging are tightly related, since global levels of 5-methylcytosine tend to decrease as aging occurs (Barbot et al., 2002; Bollati et al., 2009; Fuke et al., 2004; Nilsen et al., 2016; Singhal et al.,1987). However, this process of hypomethylation does not occur homogenously in the entire genome, and hypermethylation in promoters of several genes and other specific regions has been reported (Fraga & Esteller, 2007; Issa, 2003). In our

study, all five sites with significant results showed positive trends with aging (hypermethylation), including the CpG sites located in the gene *TET2*, thus producing different results than in humpbacks and minke whales but in concordance with those obtained in humans (Grönniger et al., 2010). Although our results indicated significant correlations with age for several CpG sites, trends found in fin whales were less consistent and with smaller R^2 values than those found in humpbacks (Polanowski et al., 2014).

Even though significant correlations were found in several CpG sites, there is one remarkable limitation in our study that deserves discussion. Standard deviations between PCR replicates were relatively high (mean around 2%) in some CpG sites, while the differences among individual samples hardly ever exceed 5%. These differences are in accordance with the technical accuracy and reproducibility of pyrosequencing when analyzing samples from different PCR reactions (Kurdyukov & Bullock, 2016; Tost & Gut, 2007). Unfortunately, total variation between individuals was small (Table 1), especially for those sites located in *CDKN2A* and *GRIA2*. This brings forward the necessity to screen for new markers associated with age that exhibit higher variability between individuals in order to reduce the effect of the analytical error. Therefore, before a widespread use of this kind of assay in fin whales, we recommend the use of Next Generation Sequencing techniques to perform a wider screening, as suggested by Goto et al. (2020).

The standard deviation of the mean difference between known and predicted ages was 2.94, similar to the results obtained in bats (Wright et al., 2018) and humpbacks (Polanowski et al., 2014), and lower than in minke whales (Goto et al., 2020). However, the *y*-intercept and the slope of the regression indicated that our model overestimates the age of young whale's and, importantly, highly underestimates the age of older fin whales, similar to the results obtained by Goto et al. (2020). In addition, when we investigated the correlation between age and each individual CpG site, we found that this relation was not totally maintained when data were split into populations. In four out of five CpG sites, correlation with age was significant in the Spanish population, but only in two out of five CpG sites was there a significant correlation in the Icelandic population.

We hypothesize that both problems, the low accuracy for estimating the age of older fin whales and the dissimilar results between populations, may be due to the lack of very old individuals, especially in the Icelandic population. In fin whales, longevity has not been properly estimated, but individuals around 80–90 years old have been reported (Aguilar & García-Vernet, 2018). In order to obtain robust calibrations, it would be highly desirable to analyze a higher number of samples from old individuals, because in our data set most of the oldest animals were 25–30 years. Indeed, most of the animals caught both in Spain and Iceland during the 1980s were younger than 30 years old and the most frequent age classes were 5–7 years old in Spain and 4–9 years old in Iceland (Aguilar & Lockyer, 1987; IWC, 2009).

While some of the differences observed between the results of the two populations may be explained by the dissimilar age range in the two data sets, in the second CpG site of *TET2* (*TET2_CpG + 21*) we detected subtle but significant differences regardless of the age of the animals. Changes in patterns of methylation along individuals' lifetime may not only be affected by intrinsic factors, such as genetic variants, but also by biological and extrinsic factors, such as aging and environmental effects, respectively (Feil & Fraga, 2012; Fraga et al., 2005). In humans, for example, several studies have reported correlation between methylation levels in different markers and environmental factors such as sun exposure (Grönniger et al., 2010), short-term changes in diet (Jacobsen et al., 2012), exercise (Barrès et al., 2012), early-life stress factors (Naumova et al., 2012), and socioeconomic status (Lam et al., 2012; McGuiness et al., 2012).

Regarding ecology, epigenetics can provide an answer to rapid adaptations to the environment, promoting a high phenotypic plasticity in front of a constantly changing environment (Verhoeven et al., 2016). Therefore, methylation patterns may play an important role by linking environmental cues and phenotypes. In addition, a potential transgenerational transmission of methylation patterns may have an important role in evolutionary processes (Jablonka & Raz, 2009; Verhoeven et al., 2016).

Several researchers have observed correlations between methylation patterns and different environmental exposures. For example, resource availability may shape differences in the epigenome of two groups of wild baboons with different foraging strategies (Lea et al., 2016). Similarly, epigenetic differences have been detected between

urban and rural populations of Darwin's finches (McNew et al., 2017). Methylation patterns have been related not only to different foraging strategies, but also to different migratory strategies (Baerwald et al., 2016). Other studies in different species have established associations between methylation patterns and other environmental factors, such as contamination (Nilsen et al., 2016), early life stress (Moghadam et al., 2017) or domestication processes (Koch et al., 2016). Therefore, we suggest that differences detected in *TET2_CpG + 21* between Icelandic and Spanish populations could be a result of the distinct environmental cues affecting both populations.

In the North Atlantic Ocean, the International Whaling Commission (IWC, 2009) currently considers seven fin whale stocks that have been mainly identified according to the location of the feeding grounds occupied during the summer. Since whales reproduce during the winter in different areas, it is of the highest relevance to establish the degree of mixing between animals that belong to each stock. In general, low levels of genetic divergence have been detected between fin whales sampled in different feeding areas (Pampoulie & Daníelsdóttir, 2013) maybe reflecting some degree of mixing in the breeding grounds. However, fin whales inhabiting waters off western Iceland and northwestern Spain seem to belong to different breeding grounds, implying a low degree of mixing between both populations. Such segregation appears to be supported by previous studies using different approaches, such as internal tagging (Gunnlaugsson & Sigurjónsson, 1989), internal and external morphology (Jover, 1992; Lockyer, 1982; Víkingsson, 1992), stable isotope analysis (Vighi et al., 2016), trace element concentrations (Sanpera et al., 1996), and other miscellaneous sources of information (Víkingsson & Gunnlaugsson, 2006). The stock of fin whales feeding off northwestern Spain is assumed to be composed of animals reproducing in a single breeding ground (IWC, 2009), which although of unidentified location, it may be located off eastern Africa (Vighi et al., 2016). Conversely, the stock summering off western Iceland appears to be composed of animals wintering in either the central Atlantic (around the Azores) or off eastern Canada (IWC, 2009; Silva et al., 2013). Whatever the case, more studies are needed to determine if differences associated with breeding grounds and migration patterns of the two groups of fin whales are associated not only to a differential genetic pool but also to different environmental pressures that in turn may cause differences in their epigenomic profiling. Again, we suggest that a wider screening of new CpG sites, based on Next Generation Sequencing techniques, should shed some light on this topic.

ACKNOWLEDGMENTS

The samples were collected at the whaling stations of IBSA (Spain) and Hvalur H/F (Iceland). The authors are grateful to Christina H. Lockyer for her contribution to the earplug readings, to the personnel of the PEBC platform (Bellvitge Biomedical Research Institute, IDIBELL) where the PCR products were analyzed, and to Dr. Concepción Arenas for comments that improved the statistical analysis. The study was part of project CGL2015-70468-R funded by the Ministerio de Economía y Competitividad of Spain and the Fondo Europeo de Desarrollo Regional (FEDER). R.G.-V.'s work was funded through a PhD APIF scholarship granted by the University of Barcelona. R.G.-V. wants to thank the Spanish State Public Employment Service (SEPE) for its financial support during the review process.

AUTHOR CONTRIBUTIONS

Raquel García-Vernet: Formal analysis; investigation; writing-original draft. **Berta Martín:** Formal analysis; supervision; writing-review & editing. **Miguel Peinado:** Supervision; writing-review & editing. **Gísli Víkingsson:** Resources; writing-review & editing. **Marta Riutort:** Resources; supervision; writing-review & editing. **Alex Aguilar:** Conceptualization; funding acquisition; supervision; writing-review & editing.

DISCLOSURE STATEMENT

M.A.P. is cofounder and equity holder of Aniling, a biotech company with no interests in this paper. M.A.P.'s laboratory has received research funding from Celgene. The rest of the authors declare no conflict of interest.

ORCID

Raquel García-Vernet https://orcid.org/0000-0001-6717-1654

REFERENCES

Aguilar, A., & Borrell, A. (1994). Assessment of organochlorine pollutants in cetaceans by means of skin and hypodermic biopsies. In M. C. Fossi & C. Leonzio (Eds.), *Nondestructive biomarkers in vertebrates* (pp. 245–267).

Aguilar, A., & García-Vernet, R. (2018). Fin whale. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), *Encyclopedia of marine mammals* (Third ed., pp. 368–3714). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-12-373553-9.00102-4

Aguilar, A., & Lockyer, C. H. (1987). Growth, physical maturity, and mortality of fin whales (*Balaenoptera physalus*) inhabiting the temperate waters of the northeast Atlantic. *Canadian Journal of Zoology*, *65*(2), 253–263. https://doi.org/10.1139/z87-040

Aguilar, A., & Nadal, J. (1984). Obtención de biopsias hipodérmicas de cetáceos en libertad [Obtaining hypodermic biopsies of cetaceans in the wild]. *Investigación Pesquera*, *48*(1), 23–29.

Árnason, Ú., Lammers, F., Kumar, V., Nilsson, M. A., & Janke, A. (2018). Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow. *Science Advances*, *4*, eaap9873. https://doi.org/10.1126/sciadv.aap9873

Baerwald, M. R., Meek, M. H., Stephens, M. R., Nagarajan, R. P., Goodbla, A. M., Tomalty, K. M. H., Thorgaard, G. H., May, B., & Nichols, K. M. (2016). Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. *Molecular Ecology*, *25*(8), 1785–1800. https://doi.org/10.1111/mec.13231

Barbot, W., Dupressoir, A., Lazar, V., & Heidmann, T. (2002). Epigenetic regulation of an IAP retrotransposon in the aging mouse: Progressive demethylation and de-silencing of the element by its repetitive induction. *Nucleic Acids Research*, *30*(11), 2365–2373. https://doi.org/10.1093/nar/30.11.2365

Barlow, J., & Clapham, P. J. (1997). A new birth-interval approach to estimating demographic parameters of humpback whales. *Ecology*, *78*(2), 535–546. https://doi.org/10.1890/0012-9658(1997)078[0535:ANBIAT]2.0.CO;2

Barrès, R., Yan, J., Egan, B., Treebak, J. T., Rasmussen, M., Fritz, T., Caidahl, K., Krook, A., O'Gorman, D. J., & Zierath, J. R. (2012). Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metabolism*, *15*(3), 405–411. https://doi.org/10.1016/j.cmet.2012.01.001

Bekaert, B., Kamalandua, A., Zapico, S. C., Van De Voorde, W., & Decorte, R. (2015). Improved age determination of blood and teeth samples using a selected set of DNA methylation markers. *Epigenetics*, *10*(10), 922–930. https://doi.org/10.1080/15592294.2015.1080413

Bird, A. P., & Wolffe, A. P. (1999). Methylation-induced repression—belts, braces, and chromatin. *Cell*, *99*(5), 451–454. https://doi.org/10.1016/S0092-8674(00)81532-9

Bocklandt, S., Lin, W., Sehl, M. E., Sánchez, F. J., Sinsheimer, J. S., Horvath, S., & Vilain, E. (2011). Epigenetic predictor of age. *PLoS ONE*, *6*(6), e14821. https://doi.org/10.1371/journal.pone.0014821

Bollati, V., Schwartz, J., Wright, R., Litonjua, A., Tarantini, L., Suh, H., Sparrow, D., Vokonas, P., & Baccarelli, A. (2009). Decline in genomic DNA methylation through aging in a cohort of elderly subjects. *Mechanisms of Ageing and Development*, *130*(4), 234–239. https://doi.org/10.1016/j.mad.2008.12.003

Booth, L. N., & Brunet, A. (2016). The aging epigenome. *Molecular Cell*, *62*(5), 728–744. https://doi.org/10.1016/j.molcel.2016.05.013

Chittleborough, R. G. (1959). Determination of age in the humpback whale, *Megaptera nodosa* (Bonnaterre). Australian *Journal of Marine and Freshwater Research*, *10*, 125–143.

Christensen, I. (1995). Interpretation of growth layers in the periosteal zone of tympanic bulla from minke whales *Balaenoptera acutorostrata*. *Developments in Marine Biology*, *4*(C), 413–423. https://doi.org/10.1016/S0163-6995(06)80043-0

Clapham, P. J., & Baker, C. S. (2018). Modern whaling. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), *Encyclopedia of marine mammals* (Third ed., pp. 1070–1074). Academic Press, Elesvier. https://doi.org/10.1016/b978-0-12-804327-1.00272-7, Whaling, Modern

Clapham, P. J., & Mattila, D. K. (1993). Reactions of humpback whales to skin biopsy sampling on a West Indies breeding ground. *Marine Mammal Science*, *9*(4), 382–391. https://doi.org/10.1111/j.1748-7692.1993.tb00471.x

D'Aquila, P., Rose, G., Bellizzi, D., & Passarino, G. (2013). Epigenetics and aging. *Maturitas*, *74*(2), 130–136. https://doi.org/10.1016/j.maturitas.2012.11.005

De Paoli-Iseppi, R., Deagle, B. E., McMahon, C. R., Hindell, M. A., Dickinson, J. L., & Jarman, S. N. (2017). Measuring animal age with DNA methylation: From humans to wild animals. *Frontiers in Genetics*, *8*, 2010–2017. https://doi.org/10.3389/fgene.2017.00106

Deaton, A. M., & Bird, A. (2011). CpG islands and the regulation of transcription. *Genes & Development*, *25*(10), 1010–1022. https://doi.org/10.1101/gad.2037511.1010

Dunshea, G., Duffield, D., Gales, N., Hindell, M., Wells, R. S., & Jarman, S. N. (2011). Telomeres as age markers in vertebrate molecular ecology. *Molecular Ecology Resources*, *11*(2), 225–235. https://doi.org/10.1111/j.1755-0998.2010.02976.x

Eipel, M., Mayer, F., Arent, T., Ferreira, M. R. P., Birkhofer, C., Gerstenmaier, U., Costa, I. G., Ritz Timme, S., & Wagner, W. (2016). Epigenetic age predictions based on buccal swabs are more precise in combination with cell type-specific DNA methylation signatures. *Aging*, *8*(5), 1034–1048. https://doi.org/10.18632/aging.100972

Feil, R., & Fraga, M. F. (2012). Epigenetics and the environment: Emerging patterns and implications. *Nature Reviews Genetics*, *13*(2), 97–109. https://doi.org/10.1038/nrg3142

Flores, K. B., Wolschin, F., & Amdam, G. V. (2013). The role of methylation of DNA in environmental adaptation. *Integrative and Comparative Biology*, *53*(2), 359–372. https://doi.org/10.1093/icb/ict019

Foulkes, W. D., Flanders, T. Y., Pollock, P. M., & Haywardt, N. K. (1997). The CDKN2A (p16) gene and human cancer. *Molecular Medicine*, *3*(1), 5–20.

Fraga, M. F., & Esteller, M. (2007). Epigenetics and aging: The targets and the marks. *Trends in Genetics*, *23*(8), 413–418. https://doi.org/10.1016/j.tig.2007.05.008

Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., Heine-Suñer, D., Cigudosa, J. C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T. D., Wu, Y., … Esteller, M. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(30), 10604–10609. https://doi.org/10.1073/pnas.0500398102

Fuke, C., Shimabukuro, M., Petronis, A., Sugimoto, J., Oda, T., Miura, K., Miyazaki, T., Ogura, C., Okazaki Y., & Jinno, Y. (2004). Age related changes in 5-methylcytosine content in human peripheral leukocytes and placentas: An HPLC-based study. *Annals of Human Genetics*, *68*(3), 196–204. https://doi.org/10.1046/j.1529-8817.2004.00081.x

George, J. C., Bada, J., Zeh, J., Scott, L., Brown, S. E., O'Hara, T., & Suydam, R. (1999). Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. *Canadian Journal of Zoology*, *77*(4), 571–580. https://doi.org/10.1139/z99-015

Goto, M., Kitakado, T., & Pastene, L. A. (2020). A preliminary study of epigenetic estimation of age of the Antarctic minke whale *Balaenoptera bonaerensis*. *Cetacean Population Studies*, *2*, 5–14. https://doi.org/10.34331/cpops.2.1_5

Grönniger, E., Weber, B., Heil, O., Peters, N., Stäb, F., Wenck, H., Korn, B., Winnefeld, M., Lyko, F. (2010). Aging and chronic sun exposure cause distinct epigenetic changes in human skin. *PLoS Genetics*, *6*(5), 6. https://doi.org/10.1371/journal.pgen.1000971

Gunnlaugsson, T., & Sigurjónsson, J. (1989). Analysis of the North Atlantic fin whale marking data from 1979–1988 with special reference to Iceland. *Report of the International Whaling Commission*, *39*, 383–388.

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., Klotzle, B., Bibikova, M., Fan, J., Gao, Y., Deconde, R., Chen, M., Rajapakse, I., Friend, S., Ideker, T., & Zhang, K. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Molecular Cell*, *49*(2), 359–367. https://doi.org/10.1016/j.molcel.2012.10.016

Herman, D. P., Yitalo, G. M., Robbins, J., Straley, J. M., Gabriele, C. M., Clapham, P. J., Boyer, R. H., Tilbury, K. L., Pearce, R. W., & Krahn, M. M. (2009). Age determination of humpback whales *Megaptera novaeangliae* through blubber fatty acid compositions of biopsy samples. *Marine Ecology Progress Series*, *392*, 277–293. https://doi.org/10.3354/meps08249

Issa, J. P. (2003). Age-related epigenetic changes and the immune system. *Clinical Immunology*, *109*(1), 103–108. https://doi.org/10.1016/S1521-6616(03)00203-1

IWC. (2009). Report of the first intersessional RMP workshop on North Atlantic fin whales. *Journal of Cetacean Research and Management*, *11*(SUPPL.), 425–452.

Jablonka, E., & Raz, G. (2009). Transgenerational epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heredity and evolution. *Quarterly Review of Biology*, *84*(2), 131–176. https://doi.org/10.1086/598822

Jacobsen, S. C., Brøns, C., Bork-Jensen, J., Ribel-Madsen, R., Yang, B., Lara, E., Hall, E., Calvanese, V., Nilsson, E., Jørgensen, S. W., Mandrup, S., Ling, C., Fernandez, A. F., Fraga, M.F., & Vaag, A. (2012). Effects of short-term high-fat overfeeding on genome-wide DNA methylation in the skeletal muscle of healthy young men. *Diabetologia*, *55*(12), 3341–3349. https://doi.org/10.1007/s00125-012-2717-8

Koch, I. J., Clark, M. M., Thompson, M. J., Deere-Machemer, K. A., Wang, J., Duarte, L., Gnanadesikan, G. E., Mccoy, E. L., Rubbi, L., Stahler, D. R., Pellegrini, M., Ostrander, E. A., Wayne, R. K., Sinsheimer, J. S., & VonHoldt, B. M. (2016). The concerted impact of domestication and transposon insertions on methylation patterns between dogs and grey wolves. *Molecular Ecology*, *25*(8), 1838–1855. https://doi.org/10.1111/mec.13480

Jarman, S. N., Polanowski, A. M., Faux, C. E., Robbins, J., De Paoli-Iseppi, R., Bravington, M., & Deagle, B. E. (2015). Molecular biomarkers for chronological age in animal ecology. *Molecular Ecology*, *24*(19), 4826–4847. https://doi.org/10.1111/mec.13357

Jover, L. (1992). Morphometric differences between Icelandic and Spanish fin whales (*Balaenoptera physalus*). *Report of the International Whaling Commission*, *42*, 747–750.

Klose, R. J., & Bird, A. P. (2006). Genomic DNA methylation: The mark and its mediators. *Trends in Biochemical Sciences*, *31*(2), 89–97. https://doi.org/10.1016/j.tibs.2005.12.008

Koch, C. M., & Wagner, W. (2011). Epigenetic-aging-signature to determine age in different tissues. *Aging*, *3*(10), 1018–1027. https://doi.org/10.18632/aging.100395

Kurdyukov, S., & Bullock, M. (2016). DNA methylation analysis: Choosing the right method. *Biology*, *5*(1), 3. https://doi.org/10.3390/biology5010003

Lam, L. L., Emberly, E., Fraser, H. B., Neumann, S. M., Chen, E., Miller, G. E., & Kobor, M. S. (2012). Factors underlying variable DNA methylation in a human community cohort. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(Supplement 2), 17253–17260. https://doi.org/10.1073/pnas.1121249109

Lea, A. J., Altmann, J., Alberts, S. C., & Tung, J. (2016). Resource base influences genome-wide DNA methylation levels in wild baboons (*Papio cynocephalus*). *Molecular Ecology*, *25*(8), 1681–1696. https://doi.org/10.1111/mec.13436

Lee, H. Y., Jung, S. E., Oh, Y. N., Choi, A., Yang, W. I., & Shin, K. J. (2015). Epigenetic age signatures in the forensically relevant body fluid of semen: A preliminary study. *Forensic Science International: Genetics*, *19*, 28–34. https://doi.org/10.1016/j.fsigen.2015.05.014

Lockyer, C. H. (1982). Preliminary investigation of some anatomical characters of fin whale ear plugs collected from different regions of the N.E. Atlantic. *Report of the International Whaling Commission*, *32*, 101–103.

Lockyer, C. H. (1984). Age determination by means of the earplug in baleen whales. *Report of the International Whaling Commission*, *34*, 692–696.

Lubetkin, S. C., Zeh, J. E., Rosa, C., & George, J. C. (2008). Age estimation for young bowhead whales (*Balaena mysticetus*) using annual baleen growth increments. *Canadian Journal of Zoology*, *86*(6), 525–538. https://doi.org/10.1139/Z08-028

McGuinness, D., McGlynn, L. M., Johnson, P. C. D., MacIntyre, A., Batty, G. D., Burns, H., Cavanagh, J., Deans, K. A., Ford, A., McConnachie, A., McGinty, A., McLean, J. S., Millar, K., Packard, C. J., Sattar, N. A., Tannahill, C., Velupillai, Y. N., & Shiels, P. G. (2012). Socio-economic status is associated with epigenetic differences in the pSoBid cohort. *International Journal of Epidemiology*, *41*(1), 151–160. https://doi.org/10.1093/ije/dyr215

McNew, S. M., Beck, D., Sadler-Riggleman, I., Knutie, S. A., Koop, J. A. H., Clayton, D. H., & Skinner, M. K. (2017). Epigenetic variation between urban and rural populations of Darwin's finches. *BMC Evolutionary Biology*, *17*(1), 1–14. https://doi.org/10.1186/s12862-017-1025-9

Moghadam, H. K., Johnsen, H., Robinson, N., Andersen, Ø., H. Jørgensen, E., Johnsen, H. K., Bæhr, V. J., & Tveiten, H. (2017). Impacts of early life stress on the methylome and transcriptome of Atlantic Salmon. *Scientific Reports*, *7*(1), 5023. https://doi.org/10.1038/s41598-017-05222-2

Mullighan, C. G. (2009). TET2 mutations in myelodysplasia and myeloid malignancies. *Nature Genetics*, *41*(7), 766–767. https://doi.org/10.1038/ng0709-766

Naue, J., Hoefsloot, H. C. J., Mook, O. R. F., Rijlaarsdam-Hoekstra, L., van der Zwalm, M. C. H., Henneman, P., Kloosterman, A. D., & Verschure, P. J. (2017). Chronological age prediction based on DNA methylation: Massive parallel sequencing and random forest regression. *Forensic Science International: Genetics*, *31*, 19–28. https://doi.org/10.1016/j.fsigen.2017.07.015

Naumova, O. Y., Lee, M., Koposov, R., Szyf, M., Dozier, M., & Grigorenko, E. L. (2012). Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents. *Development and Psychopathology*, *24*(1), 143–155. https://doi.org/10.1017/S0954579411000605

Nikaido, M., Hamilton, H., Makino, H., Sasaki, T., Takahashi, K., Goto, M., Kanda, N., Pastene, L. A., Okada, N. (2006). Baleen whale phylogeny and a past extensive radiation event revealed by SINE insertion analysis. *Molecular Biology and Evolution*, *23*(5), 866–873. https://doi.org/10.1093/molbev/msj071

Nilsen, F. M., Parrott, B. B., Bowden, J. A., Kassim, B. L., Somerville, S. E., Bryan, T. A., Bryan, C. E., Lange, T. R., Delaney, J. P., Brunell, A. M., Long, S. E., & Guillette, L. J. (2016). Global DNA methylation loss associated with mercury contamination and aging in the American alligator (*Alligator mississippiensis*). *Science of the Total Environment*, *545–546*, 389–397. https://doi.org/10.1016/j.scitotenv.2015.12.059

Nishida, S., Goto, M., Pastene, L. A., Kanda, N., & Koike, H. (2007). Phylogenetic relationships among cetaceans revealed by Y-chromosome sequences. *Zoological Science*, *24*(7), 723–732. https://doi.org/10.2108/zsj.24.723

Olsen, E., & Sunde, J. (2002). Age determination of minke whales (*Balaenoptera acutorostrata*) using the aspartic acid racemization technique. *Sarsia*, *87*(1), 1–8. https://doi.org/10.1080/003648202753631686

Olsen, M. T., Robbins, J., Bérubé, M., Rew, M. B., & Palsbøll, P. J. (2014). Utility of telomere length measurements for age determination of humpback whales. *NAMMCO Scientific Publications*, *10*(2014). https://doi.org/10.7557/3.3194

Pampoulie, C., & Daníelsdóttir, A. K. (2013). Review on the genetic stock structure of North Atlantic fin whales (*Balaenoptera physalus*): Past, present and future. *International Whaling Commission Document*, SC/65a/RMP, 1–8.

Polanowski, A. M., Robbins, J., Chandler, D., & Jarman, S. N. (2014). Epigenetic estimation of age in humpback whales. *Molecular Ecology Resources*, *14*(5), 976–987. https://doi.org/10.1111/1755-0998.12247

Riekkola, L., Zerbini, A. N., Andrews, O., Andrews-Goff, V., Baker, C. S., Chandler, D., Childerhouse, S., Clapham, P., Dodémont, R., Donnelly, D., Friedlaender, A., Gallego, R., Garrigue, C., Ivashchenko, Y., Jarman, S., Lindsay, R., Pallin, L., Robbins, J., Steel, D., Tremlett, J., Vindenes, S., & Constantine, R. (2018). Application of a multi-disciplinary approach to reveal population structure and Southern Ocean feeding grounds of humpback whales. *Ecological Indicators*, *89*, 455–465. https://doi.org/10.1016/j.ecolind.2018.02.030

Rocha, R. C., Clapham, P. J., & Ivashchenko, Y. V. (2014). Emptying the oceans: A summary of industrial whaling catches in the 20th century. *Marine Fisheries Review*, *76*(4), 37–48. https://doi.org/10.7755/MFR.76.4.3

Sanpera, C., González, M., & Jover, L. (1996). Heavy metals in two populations of North Atlantic fin whales (*Balaenoptera physalus*). *Environmental Pollution*, *91*(3), 299–307.

Sasaki, T., Nikaido, M., Hamilton, H., Goto, M., Kato, H., Kanda, N., Pastene, L. A., Cao, Y., Fordyce, R. E., Hasegawa, M., & Okada, N. (2005). Mitochondrial phylogenetics and evolution of mysticete whales. *Systematic Biology*, *54*(1), 77–90. https://doi.org/10.1080/10635150590905939

Sen, P., Shah, P. P., Nativio, R., & Berger, S. L. (2016). Epigenetic mechanisms of longevity and aging. *Cell*, *166*(4), 822–839. https://doi.org/10.1016/j.cell.2016.07.050

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*(10), e76507. https://doi.org/10.1371/journal.pone.0076507

Singhal, R. P., Mays-Hoopes, L. L., & Eichhorn, G. L. (1987). DNA methylation in aging of mice. *Mechanisms of Ageing and Development*, *41*(3), 199–210. https://doi.org/10.1016/0047-6374(87)90040-6

Smith, Z. D., & Meissner, A. (2013). DNA methylation: Roles in mammalian development. *Nature Reviews Genetics*, *14*(3), 204–220. https://doi.org/10.1038/nrg3354

Solary, E., Bernard, O. A., Tefferi, A., Fuks, F., & Vainchenker, W. (2014). The Ten-Eleven Translocation-2 (TET2) gene in hematopoiesis and hematopoietic diseases. *Leukemia*, *28*(3), 485–496. https://doi.org/10.1038/leu.2013.337

Tanabe, A., Shimizu, R., Osawa, Y., Suzuki, M., Ito, S., Goto, M., Pastene, L. A., Yoshihiro, F., & Sahara, H. (2020). Age estimation by DNA methylation in the Antarctic minke whale. *Fisheries Science*, *86*(1), 35–41. https://doi.org/10.1007/s12562-019-01371-7

Tost, J., & Gut, I. G. (2007). DNA methylation analysis by pyrosequencing. *Nature Protocols*, *2*(9), 2265–2275. https://doi.org/10.1038/nprot.2007.314

Verhoeven, K. J. F., VonHoldt, B. M., & Sork, V. L. (2016). Epigenetics in ecology and evolution: What we know and what we need to know. *Molecular Ecology*, *25*(8), 1631–1638. https://doi.org/10.1111/mec.13617

Vidal-Bralo, L., Lopez-Golan, Y., & Gonzalez, A. (2016). Simplified assay for epigenetic age estimation in whole blood of adults. *Frontiers in Genetics*, *7*, 1–7. https://doi.org/10.3389/fgene.2016.00126

Vighi, M., Borrell, A., & Aguilar, A. (2016). Stable isotope analysis and fin whale subpopulation structure in the eastern North Atlantic. *Marine Mammal Science*, *32*(2), 535–551. https://doi.org/10.1111/mms.12283

Víkingsson, G. A. (1992). Morphometrics of fin whales off Iceland and Spain. Report of the Comprehensive Assessment Special Meeting on North Atlantic Fin Whales. Annex D. *Report of the International Whaling Commission*, *42*, 611.

Víkingsson, G., & Gunnlaugsson, T. (2006). *Stock structure of fin whales* (Balaenoptera physalus) *in the North Atlantic – Indications from non-genetic data*. Document IWC/SC/57/PFI3. Available from the International Whaling Commission, https://iwc.int/home

Weidner, C. I., Lin, Q., Koch, C. M., Eisele, L., Beier, F., Ziegler, P., Bauerschlag, D. O., Jöckel, K., Erbel, R., Mühleisen, T. W., Zenke, M., Brümmendorf, T. H., & Wagner, W. (2014). Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biology*, *15*(2), R24. https://doi.org/10.1186/gb-2014-15-2-r24

Wright, P. G. R., Mathews, F., Schofield, H., Morris, C., Burrage, J., Smith, A., Dempster, E. L., & Hamilton, P. B. (2018). Application of a novel molecular method to age free-living wild Bechstein's bats. *Molecular Ecology Resources*, *18*(6), 1374–1380. https://doi.org/10.1111/1755-0998.12925

Yasunaga, G., Pastene, L. A., Bando, T., Hakamada, T., & Fujise, Y. (2017). Age estimation of Antarctic minke whales *Balaenoptera bonaerensis* based on aspartic acid racemization technique. *Fisheries Science*, *83*(6), 947–954. https://doi.org/10.1007/s12562-017-1122-0

Zhao, R., Choi, B. Y., Lee, M. H., Bode, A. M., & Dong, Z. (2016). Implications of genetic and epigenetic alterations of CDKN2A (p16INK4a) in cancer. *EBioMedicine*, *8*(127), 30–39. https://doi.org/10.1016/j.ebiom.2016.04.017

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: García-Vernet R, Martín B, Peinado MA, Víkingsson G, Riutort M, Aguilar A. CpG methylation frequency of *TET2*, *GRIA2*, and *CDKN2A* genes in the North Atlantic fin whale varies with age and between populations. *Mar Mam Sci.* 2021;1–15. https://doi.org/10.1111/mms.12808

CAPÍTULO 5

A preliminary analysis of fin whale methylome shows migration-related epigenetic differences between two North Atlantic populations

Autores: **Raquel García-Vernet**[ab], Cristian Cuevas[b], Alex Aguilar[a], Asunción Borrell[a], Marta Riutort[b]

[a] *Departamento de Biología Evolutiva, Ecología i Ciencias Ambientales, y IRBio, Facultad de Biología, Universidad de Barcelona*

[b] *Departamento de Genética, Microbiología y Estadística, y IRBio, Facultad de Biología, Universidad de Barcelona*

The fin whale is one of the most abundant baleen whales inhabiting the North Atlantic, but its current population structure is still far from being completely understood. The International Whaling Commission recognizes seven different North Atlantic populations, but genetic studies suggest high levels of gene flow among them. Here, we examined genome-scale DNA methylation in two North Atlantic fin whale populations, those off western Iceland and northwestern Spain. Although there is low genetic divergence between both populations, non-genetic markers point towards isolation of fin whales from these two areas.

In this study, we identified 215 differentially methylated regions (DMRs), of which 94 were associated with annotated genes. More than 10% of these genes were directly related to the circadian clock or other migration-related traits, such as muscle development or muscle metabolism, highlighting differences between migration patterns of both populations. We also detected two genes related to differential contamination exposure, which had been previously described between individuals from Iceland and Spain.

Overall, this preliminary study shows that epigenomics has a great potential to detect adaptative strategies to differential environments and life histories, even between populations with no evident genetic divergence.

A preliminary analysis of fin whale methylome shows migration-related epigenetic differences between two North Atlantic populations

Raquel García-Vernet[1,2], Cristian Cuevas[2], Alex Aguilar[1], Asunción Borrell[1], Marta Riutort[2]

[1] *Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain*

[2] *Institute of Biodiversity Research (IRBio) and Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain*

ABSTRACT

The fin whale is one of the most abundant baleen whales inhabiting the North Atlantic, but its current population structure is still far from being completely understood. The International Whaling Commission recognizes seven different North Atlantic populations, but genetic studies suggest high levels of gene flow among them. Here, we examined genome-scale DNA methylation in two North Atlantic fin whale populations, those off western Iceland and northwestern Spain. Although there is low genetic divergence between both populations, non-genetic markers point towards isolation of fin whales from these two areas. In this study, we identified 215 differentially methylated regions (DMRs), of which 94 were associated with annotated genes. More than 10% of these genes were directly related to the circadian clock or other migration-related traits, such as muscle development or muscle metabolism, highlighting differences between migration patterns of both populations. We also detected two genes related to differential contamination exposure, which had been previously described between individuals from Iceland and Spain. Overall, this preliminary study shows that epigenomics has a great potential to detect adaptative strategies to differential environments and life histories, even between populations with no evident genetic divergence.

INTRODUCTION

Understanding population connectivity is critical to implement proper conservation strategies to protect biodiversity (Kool *et al*., 2013). However, acquiring this kind of information may be challenging in dynamic habitats with no clear boundaries, such as marine environments (Lourie and Vincent, 2004). Complexity increases even more when

studying highly mobile vertebrate species, such as baleen whales. Application of molecular genetics to biodiversity conservation can provide valuable information about connectivity and potential reproductive isolation between populations, helping to design appropriate management measures (Frankham, 2010; Coates *et al*., 2018). Specifically, use of genetic data offers a great opportunity to identify management units, which are defined as groups of individuals whose population dynamics are demographically independent and do not rely on immigration of other individuals (Palsboll *et al*., 2007; Taylor *et al*., 2010). However, defining management units is not always trouble-free, especially when some degree of individual dispersion exists, which may lead to assigning different populations to the same management unit (Palsboll *et al*., 2007).

The fin whale (*Balaenoptera physalus*) is a cosmopolitan species and one of the most abundant baleen whales inhabiting the North Atlantic. Like other baleen whales, fin whales undertake annual migrations alternating high-latitude feeding grounds in summer and low-latitude breeding grounds in winter (Aguilar and García-Vernet, 2018). Largely based on catch and marking data (Donovan, 1991), the International Whaling Commission (IWC) recognized seven different North Atlantic management units or populations (IWC 2007). Because most of the information was collected in the feeding grounds, these management units mainly reflect the summer population structure. However, the winter breeding grounds, as well as the real connectivity among individuals from different feeding grounds, is still unknown (IWC 2007; Aguilar and García-Vernet, 2018).

Genetic-based studies have tried to shed some light on these questions, but their results are difficult to interpret (reviewed in Pampoulie *et al*., 2013). Based on mitochondrial and nuclear loci, fin whales from different North Atlantic feeding grounds showed low genetic divergence, suggesting “isolation-by-distance” among individuals from the different management units (Berubé *et al*., 1998). Later research suggests high levels of gene flow among fin whales from different feeding areas, highlighting the importance of immigration in these populations (Palsboll *et al*., 2004; Berubé *et al*., 2006). Altogether, this type of study has revealed a complex scenario which is still far from being understood.

Despite the low genetic divergence detected throughout the North Atlantic, differences among individuals inhabiting waters off western Iceland and northwestern Spain have been detected in numerous studies. Isolation between these two populations has been supported through morphological studies (Jover *et al.,* 1992), contaminant levels (Sanpera *et al.,* 1996; Vighi *et al.,* 2017), stable isotopes (Vighi *et al.,* 2016), and

epigenetics (García-Vernet *et al.,* 2021). Therefore, although connectivity among populations is still under debate, these studies seem to point out a differential life history between individuals belonging to these two populations.

The study of epigenetic modifications, like DNA methylation, offers an opportunity to better understand the ecology and biological evolution of species and populations (Herrel *et al.,* 2020), and it has become a promising tool in the conservation biology field (Walters and Schwartz, 2020). In vertebrates, methylation usually occurs on the cytosines of cytosine-phosphate-guanine (CpG) dinucleotides and is associated with transcriptional repression of nearby genes (Bird and Wolffe, 1999; Klose and Bird, 2006). Specifically, it was demonstrated that some environmentally induced changes in DNA methylation can affect gene expression, providing heterogeneity even within genetically homogenous populations (Flores *et al.,* 2013), allowing organisms to adapt to sudden changes in their environment (Rey *et al.,* 2020; Angers *et al.,* 2020; Herrel *et al.,* 2020). Among others, methylation modifications driven by the environment have been associated with differences in contaminant exposure (Nilsen *et al.,* 2016; Mancia *et al.,* 2021), migration (Baerwald *et al.,* 2016), foraging strategies (Lea *et al.,* 2016; McNew *et al.,* 2017), and salinity (Artemov *et al.,* 2017). Therefore, variation in DNA methylation could provide further information about local environmental adaptations and connectivity between populations, even among individuals with scarce genetic differentiation or relatively high gene flow (Massicotte *et al.,* 2011; Flores *et al.,* 2013; Meröndun *et al.,* 2019; Rey *et al.,* 2020).

In this study, we examined epigenomic differences between two fin whale populations, those off western Iceland and northwestern Spain. We also evaluated if these epigenetic variations were associated with genes related to the phenotypic and behavioral plasticity detected between these two populations. The main goal was to assess the potential use of epigenomics to detect differential adaptations among populations with scarce genetic divergence, which may be a useful tool to delimitate appropriate management units.

MATERIALS AND METHODS

1. Sample collection

The skin samples analyzed in this study were collected from fin whales caught by commercial whaling operations at two feeding grounds in the North Atlantic, which are managed as separate units by the International Whaling Commission (IWC, 2009). In the first, located off the northwestern coast of Spain (from hereon in referred to as the “Spanish population”), the samples were collected at the Caneliñas land factory from 11 individuals caught during the 1985 summer season. In the second, located off the

western coast of Iceland (from hereon in referred to as the “Icelandic population”), the samples were collected at the Hvalur H/F (Hafnarfjordur) land factory from eight individuals caught during the summer of 1986. All skin samples were collected by A.A. and A.B., always from the central part of the dorsal region of the body, to avoid epigenetic variation due to different sampling positions (Goto *et al.,* 2020). Immediately after collection, samples were frozen at −20ºC and preserved under this condition until the analyses were conducted.

Other factors apart from the environment can also affect methylation levels. For example, aging is known to strongly affect methylation patterns (Jung and Pfeifer 2015), and this effect has been detected in three fin whale genes (García-Vernet *et al.,* 2021). To reduce this effect, samples selected from both populations were of similar mean age (11.5 years for Spain, 12.9 years for Iceland) and similar age ranges (4 to 19 years for Spain, 8 to 20 years for Iceland). The individual’s age was determined by counting the growth layers present on a longitudinal section of their ear-plug core and following the methods described by Aguilar and Lockyer (1987).

2. DNA extraction and Reduced Representation Bisulfite Sequencing

DNA was isolated using the Blood & Cell Culture DNA Mini Kit (Qiagen). DNA concentration of each sample was quantified and qualified using a QuBit fluorometer (Thermo Fisher). Libraries were prepared using the Premium RRBS kit (Diagenode) at the “Centres Científics i Tecnològics” of the University of Barcelona (CCiT-UB). Briefly, genomic DNA (400 ng) was digested with MspI and DNA fragments were end-repaired and ligated to the adaptor barcodes. After these steps, size selection was performed using Mag-Bind Total Pure NGS beads, selecting fragments of 150 bp and higher.

Each sample was quantified performing a qPCR with a Light Cycler LC480 II. Samples with similar Ct (cycle threshold) values from the qPCR and different barcodes were pooled together, with a maximum of six samples per pool. All pools were bisulfite converted and an additional qPCR was performed to determine the optimal cycle number for PCR enrichment. We performed PCR enrichment and clean-up. Quality was assessed by calculating the concentration of each library, which was determined using a QuBit fluorometer and by determining the profiles of each library using a DNA High Sensitivity chip (Bioanalyzer). Libraries were sequenced using an Illumina HiSeq 2000 (50bp single read ends, six samples per lane) at the CRG (Centre for Genomic Regulation, Barcelona). Initial quality checks were carried out with FASTQC (Andrews 2010). Trim Galore! (https://github.com/FelixKrueger/TrimGalore) was used to perform adapter and quality trimming, using the “- - rrbs” flag option.

3. Alignment and differentially methylated CpGs

At present, genomes of several species from the Balaenopteridae family are available (Yim *et al.,* 2014, Árnason *et al.,* 2018, Tollis *et al.,* 2019). However, only two of these genomes have been assembled *de novo* and properly annotated: those of the minke whale (*Balaenoptera acutorostrata*) and of the humpback whale (*Megaptera novaeangliae*). Both species are phylogenetically close to the fin whale: minke and fin whales diverged around 10.5 million years ago ago, while humpback and fin whales diverged around 5 million years ago (Árnason *et al.,* 2018). Therefore, both genomes were *in silico* bisulfite-converted using the Bismark v. 0.23.1 software (Krueger and Andrews 2011) to perform an independent alignment of our reads to each genome.

Reads of each sample were aligned to both minke and humpback whale genomes using the default parameters of Bowtie2 (Langmead and Salzberg 2012) implemented in Bismark. Reads which were ambiguously mapped were removed from the subsequent analyses. We extracted the percentage of methylation for each cytosine using the Bismark methylation extractor script, and we later obtained a genome-wide cytosine methylation report using the module coverage2cytosine. Both scripts were implemented in Bismark.

To ensure better quality downstream analyses, a minimum coverage of 10 reads per cytosine in at least six individuals per population was required to consider a CpG position for the analysis. The R package Methylkit (Akalin *et al.,* 2012) was used to identify differentially methylated bases between the Icelandic and Spanish populations. During the identification of differentially methylated cytosines (DMC), we defined cutoffs based on q-value (q-value < 0.01) and on percentage methylation difference (> 25%).

We identified differentially methylated regions (DMRs) using the eDMR R package (Li *et al.,* 2013), which can be used as an expansion of the Methylkit R package. Following the previous settings, individual CpG positions had to present at least a 25% methylation difference with a q-value < 0.01 to be considered a DMC. To be considered a DMR, regions should contain at least 3 CpG with 1 DMC, and with an absolute mean methylation difference >20%. Again, a minimum of six individuals per population were required to consider a CpG position for the analysis.

4. Gene annotation and gene ontology

DMRs were annotated via BEDOPS (Neph *et al.,* 2012), using the already published annotations of minke (https://www.ncbi.nlm.nih.gov/) and humpback whales

(https://doi.org/10.7910/DVN/ADHX1O). We annotated all genes that were at <1000 bp of each DMR.

The latter analyses were performed using the results aligned to the minke whale genome. We used GeneCards (https://www.genecards.org/) to investigate the functions of the genes associated with all DMRs. For the 30 DMRs with lowest q-value, we visualized their differential methylation pattern across individuals and performed a hierarchical cluster analysis using the "heatmap" function with default parameters. In three individuals (IC86042, SP84002, SP84083), less than half of the 30 DMRs met the minimum coverage required (5, 11, and 6 DMRs, respectively), and were, therefore, excluded from this visualization.

Finally, we ran the InterProScan command line (Jones *et al.,* 2014) using the available protein sequences of the minke whale genome (https://www.ncbi.nlm.nih.gov/) to obtain the gene ontology (GO) terms of all proteins in the minke genome. We used Gostats (Falcon 2007) to perform enrichment analysis, comparing the list of proteins associated with the DMRs against the minke gene ontology terms. We used Revigo to summarize the GO terms (Supek *et al.,* 2011).

RESULTS

Mapping, coverage, and preliminary cluster analysis

After quality filtering, the average number of reads per individual was 37.8 million (ranging from 17.1 to 76.9 million). Mapping performance was similar for both genomes. 51% of the reads mapped to the minke whale genome (range: 38% - 54 %, depending on the individual, Table S1), and 56% of the reads mapped to the humpback whale genome (range: 43 – 59%, Table S2).

After setting the minimum read coverage to 10, we obtained an average of 956,292 and 1,098,240 CpG sites for the minke and the humpback genomes, respectively (Table 1).

Differentially methylated sites and regions

A total of 79,049 and 88,280 CpG sites (when mapping to the minke and the humpback whale genomes, respectively) were recovered in at least six individuals per population. These positions were used to investigate differentially methylated sites and regions between the Icelandic and Spanish populations. CpG sites that met all fixed requirements to be considered a DMC (>25% methylation difference, < 0.01 q-value) ranged from 2,624 (minke genome) to 3,020 CpGs (humpback genome).

***Table 1**: CpGs and mean coverage of each CpG after excluding all CpGs without a minimum 10 read depth. Mapping was performed with the minke whale genome (second and third column) and humpback whale genome (fourth and fifth column)*

Sample	CpG (minke)	Mean Cov. (minke)	CpG (humpback)	Mean Cov. (humpback)
IC86008	845,903	19.8	972,357	19.1
IC86013	1,013,373	18.7	1,145,269	18.6
IC86016	784,256	39.0	908,316	37.5
IC86018	838,098	17.6	943,997	17.5
IC86027	951,722	23.9	1,090,634	22.9
IC86032	1,030,467	24.3	1,185,273	23.0
IC86042	266,047	17.9	303,864	19.4
IC86048	947,330	23.8	1,088,853	22.4
SP84002	419,324	34.9	475,206	34.6
SP84059	1,143,090	20.7	1,318,701	19.9
SP84062	789,462	20.7	905,672	20.0
SP84071	1,386,194	25.0	1,602,510	23.8
SP84073	1,974,264	29.9	2,296,148	28.5
SP84074	1,124,768	24.3	1,292,894	23.0
SP84083	454,065	34.5	518,331	33.7
SP84084	972,454	21.7	1,119,095	20.6
SP84087	801,736	21.3	914,580	20.4
SP84092	1,799,899	25.8	2,067,844	25.0
SP84093	627,096	20.6	717,025	19.9
Mean	**956,292**	**24.4**	**1,098,240**	**23.7**

Based on these DMCs, we looked for differentially methylated regions (DMR) that met our minimum requirements (contain at least 3 CpG with 1 DMC, and a global methylation difference of >20%). We found 215 and 258 DMRs for the minke and humpback genomes, respectively.

DMRs were annotated using the minke and humpback whale annotations. For the reads aligned against the minke genome, we identified 94 DMR that were associated with at least one gene (44.6% of total DMRs). However, when using the humpback whale annotation, we only identified 32 genes (12.4% of total DMRs). Of these 32 genes, 13 were also identified using minke annotations. Due to the low performance when using the humpback whale annotation, downstream analyses were performed only using DMRs that were identified and annotated using the minke whale genome.

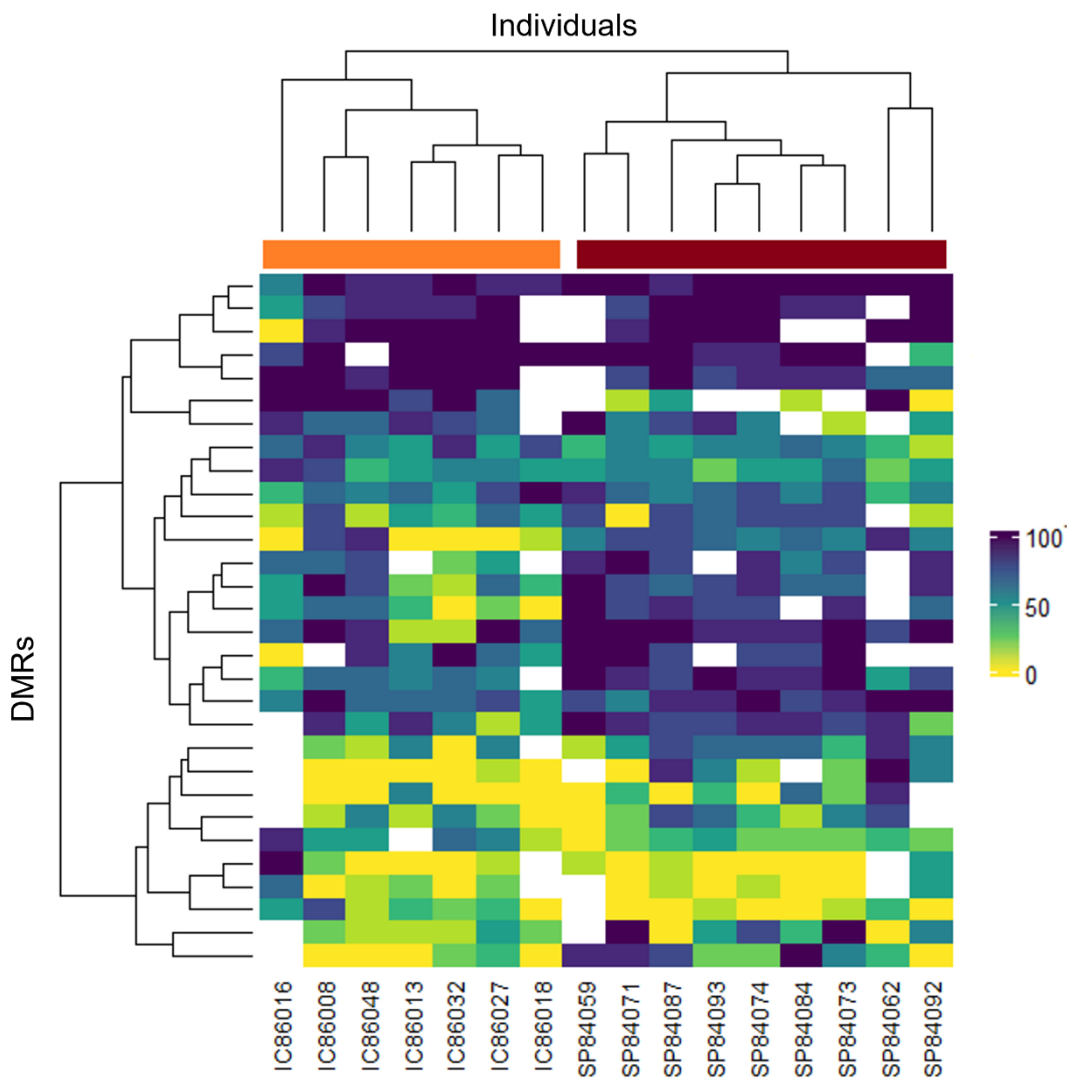


***Figure 1**: Heatmap and hierarchical cluster based on the top 30 DMR methylation percentages. Columns represent individuals from both populations (orange for Iceland, dark red for Spain).*

The 30 DMRs with the lowest q-values were visualized via a heatmap (Figure 1). Global methylation difference between populations for these DMRs ranged from 20.1 to 41.8%. Hierarchical cluster showed that individuals were grouped by population.

Gene Ontology and gene functions

We found 97 annotated genes associated with 94 different DMRs (Table S3). DMRs were mainly found on intronic (63) or exonic regions (27). Only seven DMRs were located at upstream or downstream regions, which may be due to our conservative range setting (1 kb distance). Hypermethylation of DMRs was equally distributed among all genomic features for the two populations (Figure 2). Nine of the 97 annotated genes were closely

related to the circadian clock (Table 2), which is a key element for regulating different behavioral and physiological rhythms (Reppert and Weaber, 2002; Ko and Takashi, 2006). We also found three genes related to muscle metabolism and growth, and two genes whose expression has been related to differences in exposure to contaminants (Table 2).

Finally, we investigated which gene ontology (GO) terms were significantly enriched in our DMRs. The most common GO terms were associated with regulation processes: GO:0065007 (biological regulation), GO:0050794 (regulation of cellular process), and GO:0019222 (regulation of metabolic process). A summary of our results for GO Biological Process ontology associations for DMR can be found in Table S4.

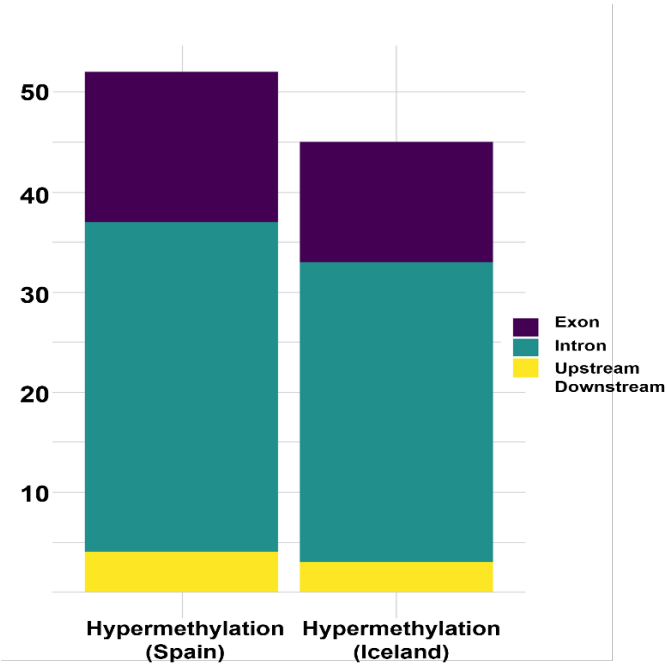


***Figure 2**: Count of genomic features which are hypermethylated in the Spanish and the Icelandic population, respectively. Each color indicates a different genomic feature. Proportions are very similar for both populations and for all genomic features.*

DISCUSSION

Here, we investigated the methylation patterns across the genome of two populations of fin whales inhabiting the North Atlantic. We investigated specific epigenomic differences between both populations and found that the top 30 differentially methylated regions (DMRs) detected was enough to separate individuals of both populations. Moreover, we identified close to 100 genes associated with these DMRs (differentially methylated regions) and, although we found a high diversity in their functions, 13 of these genes were clearly linked to interesting phenotypes.

Table 2: *DMRs associated with genes related to the circadian clock (9), muscle development (3), and contamination exposure (2), respectively. Meth diff. indicates the comparison between both populations, with positive values indicating increased methylation and negative values indicating decreased methylation in individuals from the Spanish population. CpGs indicates the total number of CpGs, and DMCs indicates the number of these CpGs presenting differential methylation between both populations. q-value indicates the significance of each DMR.*

ID	Name	Meth diff	CpGs	DMCs	q-value	Region
PER3	Period Circadian Regulator 3	-42.0	3	3	1.72E-41	Exon
MPPED1	Metallophosphoesterase Domain Containing 1	-20.1	7	3	1.92E-68	Exon
RBM4B	RNA binding motif protein 4B	-28.9	5	4	2.61E-36	Exon
GAD2	Glutamate Decarboxylase 2	-21.2	3	1	7.30E-29	Intron
CAMK2G	Calcium/Calmodulin Dependent Protein Kinase II Gamma	-23.3	3	1	7.32E-09	Intron
STK32B	Serine/Threonine Kinase 32B	-20.5	3	1	2.19E-21	Intron
RPS6KA2	Ribosomal Protein S6 Kinase A2	29.7	3	2	2.13E-17	Intron
SLC8A2	Solute Carrier Family 8 Member A2	23.1	3	1	4.15E-15	Exon
PALLD	Palladin, cytoskeletal associated protein	25.8	3	1	1.33E-15	Intron
CAPN1	Calpain 1	-22.8	3	1	1.28E-11	Intron
COL22A1	Collagen Type XXII Alpha 1 Chain	-21.7	3	1	1.54E-17	Intron
COL15A1	Collagen Type XV Alpha 1 Chain	20.1	3	1	8.24E-18	Exon
CYP26C1	Cytochrome P450 Family 26 Subfamily C Member 1	-22.3	3	1	1.95E-33	Upstream
AHRR	Aryl-Hydrocarbon Receptor Repressor	25.1	4	1	1.38E-35	Exon

Reference genome choice and sample size

To date, the fin whale genome has not been assembled *de novo*, which made it impossible to use it as our reference genome, which would have been ideal. Instead, we used the closely related minke and humpback whale genomes to map our reads. Although whale's phylogeny has not been completely resolved, it is known that rorqual

radiation occurred fast (Árnasson *et al.,* 2018). This fact indicates the phylogenetic closeness of these two species and supports the use of both minke and humpback whale genomes as reference genomes for our work with fin whale. After performing the first analyses, we decided to use only the minke genome for the remaining analyses because its annotation was more complete than that of the humpback and yielded better results. Nonetheless, based on our initial analyses, with this strategy we may be missing some DMRs, but our interest was focused on detecting which genes were related to the DMRs, rather than quantifying population differences. Hence, in the near future, when a fin whale genome becomes available and well annotated, more DMRs may be found, however current analyses have unveiled interesting results.

Sample size and storage conditions are the second limitation of this study. One prerequisite for selecting an individual's skin sample was to have information on its age, and that this age should be within a maximum range of 15 years (see Materials and Methods section). In addition, skin samples had been preserved at -20ºC for almost 40 years, which in some specific cases made DNA extraction difficult, which also reduced the final number of samples. Although similar studies have been conducted with a similar number of samples per group, most of these also controlled the experimental conditions (see for example Metzger and Schulte, 2018; Gavery *et al.,* 2019; Hearn *et al.,* 2019). In our study, samples were taken from wild individuals, which adds extra noise when comparing both populations. Therefore, our research provides some initial insights into epigenetic differences between two populations of a baleen whale species, however, the findings presented here should be taken as preliminary results.

DMRs in genes related to the circadian clock

Nine genes showing differential methylation were related to the circadian clock. Circadian clock allows us to anticipate a great variety of environmental stimuli associated with daily fluctuations, and is considered a key element for understanding animal migrations (Gwinner, 1996; Coppack, 2008; Häfker *et al.,* 2017).

We identified one DMR associated with an exon of *Period circadian regulator 3* (*PER3*) (Table 2), with the Spanish population being less methylated than the Icelandic population. *Period* genes are involved in the circadian network, although *PER3* is considered non-essential for correct circadian rhythm's maintenance (Bae *et al.,* 2001). However, it has been repeatedly linked to sleep structure and regulation in humans and animals (Viola *et al.,* 2007; Hida *et al.,* 2014; Archer *et al.,* 2018), and significant hypomethylation in the gene body has been detected in nightshift workers, suggesting an associated decreased gene expression (Bracci *et al.,* 2014; Bhatti *et al.,* 2015). In

birds, where *PER3* shows a circadian-related expression (Johnston *et al.,* 2016), it has been proposed as a candidate gene for migration phenotypes (Ruegg *et al.,* 2014), but the association of genomic variants with migratory phenotypes is still not clear (Delmore *et al.,* 2016; Johnston *et al.,* 2016; Ramos *et al.,* 2017). Therefore, our results may indicate a reduced expression of PER3 in the Spanish population, but its effect on migration it still difficult to infer.

Other DMRs were also associated with genes directly related to circadian rhythms. For instance, we found DMRs linked to *metallophosphoesterase domain containing 1* (*MPPED1*), *RNA binding motif protein 4B* (*RBM4B*), and *Glutamate Decarboxylase 2* (*GAD2*). *MMPED1* is expressed in the brain (Chen *et al.,* 2010), and although its functions are still not fully understood, it has been associated with sleep and circadian rhythm patterns (Li and Zhao, 2020). *RBM4B* is a posttranscriptional regulator of the circadian clock via activating *PER1* translation (Markus and Morris, 2009). A knockout of *RBM4B* results in altered circadian cycles in mice, highlighting its importance in the circadian network (Kojima *et al.,* 2007). GAD2 is expressed in GABAergic neurons and catalyzes the production of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Pan 2012). Among other functions, GABA has an important role in synchronizing circadian rhythms among suprachiasmatic nuclei (SCN) neurons, which regulate most of the body's circadian rhythms (Liu and Reppert, 2000; DeWoskin *et al.,* 2015; Ma and Morrison, 2021).

We found three DMRs associated with three genes encoding serine-threonine protein kinases: *Calcium/Calmodulin Dependent Protein Kinase II Gamma* (*CAMK2G*), *Serine/Threonine Kinase 32B* (*STK32B*), and *Ribosomal Protein S6 Kinase A2* (*RPS6KA2*). At the general level, proteins from the serine-threonine family catalyze the phosphorylation of serine and threonine amino acids in proteins and, among other functions, are involved in regulating the circadian clock (Eide *et al.,* 2005; Reischl and Kramer 2011). Specifically, *CAMK2G* activation has been recently detected during the sleep-wake transition, indicating a function in circadian rhythm regulation (Brüning *et al.,* 2019; Su *et al.,* 2021), and also plays a role in hippocampus-dependent learning and memory (Proietti Onori *et al.,* 2018). *RPS6KA2* has been suggested as a potentially important SCN gene, also linked to circadian rhythm regulation (McCarthy *et al.,* 2019; Beligala *et al.,* 2019).

Learning and memory also have a relevant role in shaping whale migrations (Abrahms *et al.,* 2019). In addition to *CAM2KG*, which was also related to learning and memory, we detected another gene related to these two traits: *Solute Carrier Family 8 Member*

A2 (*SLC8A2*). *SLC8A2* is involved in learning and memory consolidation (Molinaro *et al.,* 2013), and DNA methylation seems to affect its expression (Ding and Cui 2017).

We detected one last DMR located in an intron of *Palladin, cytoskeletal associated protein* (*PALLD*). It is involved in the organization of the actin cytoskeleton and, although it is apparently not related to the circadian clock, several studies have related it to migration (Baerwald *et al.,* 2016; Delmore *et al.,* 2016). Baerwald *et al.,* (2016) compared the methylome landscape between migratory and non-migratory rainbow trout and found a 24.4% increase in *PALLD* gene methylation in non-migratory individuals. In our study, fin whales from the Spanish population presented an average increase of 25.8% in methylation compared with the Icelandic population. This result could reflect a difference between both populations, where the Icelandic individuals would show a stronger migratory phenotype than Spanish individuals.

All these differences may be consequence of differential migration patterns between both populations. The fin whale population summering off Spain is assumed to be composed of individuals from a single breeding ground (IWC 2009), but their winter locations are still not clear. Whaling data from northwestern Spain showed a peak in abundance during the months of July and August, in concordance with the use of this area as a summer feeding ground. However, fin whales were also caught, in smaller numbers, in late autumn, winter, and spring (Sanpera and Aguilar, 1992). This data suggests that although most of the fin whales in this population migrate during autumn, some animals may remain in the area and/or a sequential occupation of northwestern Spain's coasts by fin whales from other feeding grounds may occur (Silva *et al.,* 2019; Gauffier *et al.,* 2020), who could use this region as a wintering ground. The bulk of the Spanish fin whale population seems to migrate to southern areas, for example off the northwestern coast of Africa (Vighi *et al.,* 2016), although some animals seem to travel into the western Mediterranean Sea (Castellote *et al.,* 2012; Gauffier *et al.,* 2018; Pereira *et al.,* 2020).

On the other hand, the population summering off Iceland seems to be composed mainly of individuals wintering off the central Atlantic, including the Azores region (IWC 2009; Silva *et al.,* 2013). In addition, recent studies suggest that fin whales sampled in the Azores during spring had been previously feeding off northwestern Spain during the winter, supporting a sequential occupation of the latter area by fin whales from different feeding grounds (Silva *et al.,* 2019; Gauffier *et al.,* 2020). Whatever the case, it seems clear that the Icelandic and the Spanish populations perform dissimilar migrations, both in distance and route, with the Icelandic population travelling longer distances through colder waters than the Spanish population. In addition, departure environmental cues,

such as photoperiod (Bani Assadi and Fraser, 2021), temperature (Burnside *et al.,* 2021), or resource tracking (Visser *et al.,* 2011; Abrahms *et al.,* 2019) may present strong differences between the Icelandic and Spanish coasts. Although distribution during winter is still not clear, fin whales seem to show fidelity to their feeding grounds (see, for example, Robbins *et al.,* 2007), so it is reasonable to expect some degree of adaptation to the specific departure environmental conditions of their preferred summer feeding region.

However, it is difficult to infer the impact that methylation patterns may have on complex aspects, such as migration. For most genes, there are no previous studies on how methylation affects their expression (see above), and although cytosine methylation is generally considered to be associated with the inhibition of gene expression (Siegfried and Simon, 2010), it is difficult to predict what effect it would have on the different migratory phenotypes. Therefore, our results show a potential link between differential migratory phenotypes and the genes mentioned above, but further studies will be required to better understand the nature of these associations.

We should also note that, although in this study we analyzed skin, most of the DMRs discussed above were associated with genes whose expression occurs primarily in the brain. Similar results were obtained when analyzing muscle from rainbow trout, where most DMRs were also associated with genes involved in neuronal development (Baerwald *et al.,* 2016). It is possible that, even though there are specific epigenetic patterns among tissues, some differences may be detected regardless of the tissue analyzed. *In vitro* studies have shown that global DNA methylation levels do not show a drastic change during differentiation (Geiman and Muegge, 2010). Transition from undifferentiated to somatic cells only requires subtle changes in DNA methylation at specific promoters (see, for example, Meissner *et al.,* 2008). In addition, the skin and the nervous system develop from the same precursor tissue, the neuroectoderm (Fuchs, 2007), potentially leading to more similarities between both tissues.

DMRs in genes related to muscle metabolism and growth

We also found three DMRs associated with genes related to muscle metabolism and growth: *Calpain 1* (*CAPN1*), *Collagen Type XXII Alpha 1 Chain* (*COL22A1*), *Collagen Type XV Alpha 1 Chain* (*COL15A1*). *CAPN1* is a calcium regulated protease and has a key role during muscle regeneration (Kemp *et al.,* 2013). Starvation seems to increase CAPN1 expression (Salem *et al.,* 2005), while hypomethylation of its promoter has been related to a reduction in its expression (Fernandez *et al.,* 2012). Although our DMR is located in an intronic zone, the relative hypomethylation found in the Spanish individuals

may suggest a lower expression in this population compared with the Icelandic population. *COL22A1* and *COL15A1* encode proteins which contain one or more collagen-like domains. *COL15A1* has a role during lesion development (Durgin *et al.,* 2017), while *COL22A1* strengthens skeletal muscle attachments during contractile activity, and some alleles with higher expression have been linked to muscle injury risk (Charvet *et al.,* 2013; Miyamoto-Mikani *et al.,* 2020). Differences related to muscle metabolism and growth may also be a consequence of the different migration patterns between both populations, especially related to the different distances traveled during migratory movements.

DMRs in genes related to contamination

The DNA methylation landscape is altered by different environmental cues, and contamination is not an exception (Nilsen *et al.,* 2016; Curtis *et al.,* 2021). Two DMRs associated with genes whose expression is related to the presence of contaminants: *Cytochrome P450 Family 26 Subfamily C Member 1* (*CYP26C1*) and *Aryl-Hydrocarbon Receptor Repressor* (*AHRR*).

CYP26C1 is a member of the cytochrome P450 superfamily. Cytochrome P450 proteins participate in a wide range of metabolic reactions and play a core role during the metabolism of several contaminants such as organochlorine compounds, including PCBs (Polychlorinated biphenyls) (Watanabe *et al.,* 1989; Boon *et al.,* 1997) and the polycyclic aromatic hydrocarbons (e.g., Wilson *et al*., 2005). In addition, heavy metal cations are capable of inducing *Cyp1a1* gene expression (Korashy *et al.,* 2005). Here, we found that the Spanish population presented an average hypomethylation of 22.3% of *CYP26C1* compared with the Icelandic population. This DMR was located 100bp upstream of the 5‘UTR exon, probably corresponding to the promoter region. In other P450 genes, hypomethylation of enhancer and promoter has been associated with higher expression of P450 (Tokizane *et al.,* 2005). In addition, Mancia *et al.,* found that *CYP26B1*, another P450 gene, was also hypomethylated in fin whales showing a high-level of contaminant exposure.

On the other hand, we found that the Spanish population showed an average hypermethylation of 25%, compared with the Icelandic population, in the DMR located in an exon of *AHRR.* AHRR mediates the toxicity of dioxin-like compounds and polycyclic aromatic hydrocarbons (e.g., Calò *et al.,* 2014; Zhou *et al.,* 2010), and its hypomethylation has been repeatedly related to smoking status, suggesting an association between increased expression and hypomethylation (see, for example,

Philibert *et al.,* 2020). Contrasting with the previous finding where P450 appears to be more expressed in the Spanish population, in this case, we observe the opposite pattern.

In general, contaminant levels in baleen whales are low compared with other marine mammals (Aguilar and García-Vernet 2018). Different concentrations of heavy metals have been detected between both populations in muscle and bone (Sanpera *et al.,* 1996; Vigh *et al.,* 2017), although in the former, concentrations were higher in Iceland and in the latter higher in Spain. PCB concentrations are quite low in both populations, being slightly lower in the Icelandic fin whales (Borrell, 1993; Aguilar and Borrell, 1988). Therefore, although different contamination patterns exist between the two populations, these patterns may differ depending on the contaminant and tissue analyzed.

Conclusions

In summary, although both populations do not present a clear genetic differentiation, we found evident epigenetic differences between them. Some of these epigenetic differences were associated with genes related to different life history traits, especially migration-related traits, suggesting that this diversity reflects differential migrations between individuals from the two feeding grounds. Overall, our results show that epigenomics has a great potential to detect adaptative strategies to differential environments and life histories, which can be useful to better discriminate among populations presenting low genetic divergence.

Acknowledgments

This study was part of project PID2020-119712GB-I00 funded by the Ministerio de Ciencia e Innovación of Spain, MCIN/AEI/10.13039/501100011033. R.G-V's work was funded through a Ph.D. APIF scholarship granted by the University of Barcelona and through the Spanish State Public Employment Service (SEPE). Authors are grateful to the staff of the MFRI who assisted in the fieldwork. Library preparation was conducted at the Centres Científics i Tecnològics (CCiT-UB) of the University of Barcelona.

References

Abrahms B., Hazen E.L., Aikens E.O., Savoca M.S., Goldbogen J.A., Bograd S.J., Jacox M.G., Irvine L.M., Palacios D.M., Mate, B.R. 2019. Memory and resource tracking drive blue whale migrations. Proceedings of the National Academy of Sciences, 116(12): 5582-5587.

Aguilar A., Lockyer C. H. 1987. Growth, physical maturity, and mortality of fin whales (*Balaenoptera physalus*) inhabiting the temperate waters of the northeast Atlantic. Canadian Journal of Zoology, 65(2): 253–263.

Aguilar A., Borrell A. 1988. Age-and sex-related changes in organochlorine compound levels in fin whales (*Balaenoptera physalus*) from the eastern North Atlantic. Marine Environmental Research, 25(3):195-211.

Aguilar A., García-Vernet R. 2018. Fin whale. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), Encyclopedia of Marine Mammals (Third Edition): 368-371

Akalin A., Kormaksson M., Li S., Garrett-Bakelman F.E., Figueroa M.E., Melnick A., Mason C.E. 2012. methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. Genome Biology, 13: R87.

Andrews S. 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data [Online]. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Angers B., Perez M., Menicucci T., Leung C. 2020. Sources of epigenetic variation and their applications in natural populations. Evolutionary Applications, 13:1262–1278.

Archer S.N., Schmidt C., Vandewalle G., Dijk, D.J. 2018. Phenotyping of PER3 variants reveals widespread effects on circadian preference, sleep regulation, and health. Sleep medicine reviews, 40: 109-126.

Árnason U., Lammers F., Kumar V., Nilsson M.A., Janke A. 2018. Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow. Science Advances, 4: eaap9873.

Artemov A.V., et al. 2017. Genome-wide DNA methylation profiling reveals epigenetic adaptation of stickleback to marine and freshwater conditions. Molecular biology and evolution, 34(9): 2203-2213.

Bae K., Jin X., Maywood E.S., Hastings M.H., Reppert S.M. Weaver D.R. 2001. Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. Neuron, 30(2): 525-536.

Baerwald M.R., Meek M.H., Stephens M.R., Nagarajan R.P., Goodbla A.M., Tomalty K.M., Thorgaard G.H., May B., Nichols K.M., 2016. Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. Molecular Ecology, 25(8): 1785-1800.

Bani Assadi S., Fraser K.C. 2021 Experimental manipulation of photoperiod influences migration timing in a wild, long-distance migratory songbird. Proceedings of Royal Society B, 288: 20211474.

Beligala D.H., De A., Malik A., Silver R., Rimu K., LeSauter J., McQuillen H.J., Geusz, M.E. 2019. Musashi-2 and related stem cell proteins in the mouse suprachiasmatic nucleus and their potential role in circadian rhythms. International Journal of Developmental Neuroscience, 75:44-58.

Berubé M., Aguilar A., Dendanto D., Larsen F., Notarbartolo di Sciara G., Sears R., Sigurjónsson J., Urban-R J., Palsboll P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. Molecular Ecology, 7: 585–599.

Berubé M., *et al*. 2006. High rates of gene flow among geographic locations in North Atlantic fin whales (*Balaenoptera physalus*). IWC/ SC/58/PFI6.

Bhatti P., Zhang Y., Song X., Makar K.W., Sather C.L., Kelsey K.T., Houseman E.A., Wang, P. 2015. Nightshift work and genome-wide DNA methylation. Chronobiology International, 32(1): 103-112.

Bird A.P., Wolffe A.P. 1999. Methylation-induced repression—belts, braces, and chromatin. Cell, 99(5): 451–454.

Boon J.P., Van der Meer J., Allchin C.R., Law R.J., Klungsøyr J., Leonards P.E.G., Spliid H., Storr-Hansen E., Mckenzie C., Wells, D.E. 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450. Archives of Environmental Contamination and Toxicology, 33(3): 298-311.

Borrell A. 1993. PCB and DDT in blubber of cetaceans from the northeastern north Atlantic. Marine Pollution Bulletin, 26(3): 146-151.

Bracci M., Manzella N., Copertaro A., Staffolani S., Strafella E., Barbaresi M., Copertaro B., Rapisarda V., Valentino M., Santarelli L. 2014. Rotating-shift nurses after a day off: peripheral clock gene expression, urinary melatonin, and serum 17-β-estradiol levels. Scandinavian journal of work, environment & health, 40(3): 295-304.

Brüning F., Noya S.B., Bange T., Koutsouli S., Rudolph J.D., Tyagarajan S.K., Cox J., Mann M., Brown S.A., Robles M.S., 2019. Sleep-wake cycles drive daily dynamics of synaptic phosphorylation. Science, 366(6462).

Burnside R.J., Salliss D., Collar N.J., Dolman P.M. 2021. Birds use individually consistent temperature cues to time their migration departure. Proceedings of the National Academy of Sciences, 118(28): e2026378118

Calò M., Licata P., Bitto A., Cascio P.L., Interdonato M., Altavilla D. 2014. Role of *AHR*, AHRR and ARNT in response to dioxin-like PCBs in *Spaurus aurata*. Environmental Science and Pollution Research, 21(24): 14226-14231.

Castellote M., Clark C.W., Lammers M.O. 2012. Fin whale (*Balaenoptera physalus*) population identity in the western Mediterranean Sea. Marine Mammal Science, 28(2): 325-344.

Charvet B., Guiraud A., Malbouyres M., *et al*. 2013. Knockdown of *col22a1* gene in zebrafish induces a muscular dystrophy by disruption of the myotendinous junction. Development, 140(22): 4602-4613.

Chen C.M., Wang H.Y., You L.R., Shang R.L., Liu F.C. 2010. Expression analysis of an evolutionary conserved metallophosphodiesterase gene, *Mpped1*, in the normal and β-catenin–deficient malformed dorsal telencephalon. Developmental Dynamics, 239(6): 1797-1806.

Coates D.J., Byrne M., Moritz C. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. Frontiers in Ecology and Evolution, 6: 165.

Coppack T., Becker S.F., Becker P.J.J. 2008. Circadian flight schedules in night-migrating birds caught on migration. Biology Letters, 4: 619–622

Curtis S.W., Cobb D.O., Kilaru V., Terrell M.L., Marder M.E., Barr D.B., Marsit C.J., Marcus M., Conneely K.N., Smith A.K. 2021. Genome-wide DNA methylation differences and polychlorinated biphenyl (PCB) exposure in a US population. Epigenetics, 16(3): 338-352.

Delmore K.E., Toews D.P., Germain R.R., Owens G.L., Irwin D.E., 2016. The genetics of seasonal migration and plumage color. Current Biology, 26(16): 2167-2173.

DeWoskin D., Myung J., Belle M.D., Piggins H.D., Takumi T., Forger D.B. 2015. Distinct roles for GABA across multiple timescales in mammalian circadian timekeeping. Proceedings of the National Academy of Sciences, 112(29): E3911-E3919.

Ding Y.X., Cui H., 2017. Integrated analysis of genome-wide DNA methylation and gene expression data provide a regulatory network in intrauterine growth restriction. Life sciences, 179: 60-65.

Donovan G.P. 1991. A review of IWC stock boundaries. Reports of the International Whaling Commission, Special Issue Series, 13: 39-68.

Durgin B.G., et al. 2017. Smooth muscle cell-specific deletion of *Col15a1* unexpectedly leads to impaired development of advanced atherosclerotic lesions. American Journal of Physiology-Heart and Circulatory Physiology, 312(5): H943-H958.

Eide E.J., Kang H., Crapo S., Gallego M., Virshup D.M. 2005. Casein kinase I in the mammalian circadian clock. Methods in enzymology, 393: 408-418.

Falcon S., Gentleman R. 2007. Using GOstats to test gene lists for GO term association. Bioinformatics, 23(2): 257-258. DOI: 10.1093/bioinformatics/btl567

Fernandez S.V., Huang Y., Snider K.E., Zhou Y., Pogash T.J., Russo J. 2012. Expression and DNA methylation changes in human breast epithelial cells after bisphenol A exposure. International journal of oncology, 41(1): 369-377.

Flores K.B., Wolschin F., Amdam G.V. 2013. The Role of Methylation of DNA in Environmental Adaptation. Integrative and Comparative Biology, 53(2): 359–372.

Frankham R. 2010. Challenges and opportunities of genetic approaches to biological conservation. Biological conservation, 143(9): 1919-1927.

Fuchs E. 2007. Scratching the surface of skin development. Nature, 445(7130): 834-842.

García-Vernet R., Martín B., Peinado M.A., Víkingsson G., Riutort M., Aguilar A. 2021. CpG methylation frequency of *TET2*, *GRIA2*, and *CDKN2A* genes in the North Atlantic fin whale varies with age and between populations. Marine Mammal Science.

Gauffier P., Verborgh P., Giménez J., Esteban R., Sierra J.M.S., de Stephanis R. 2018. Contemporary migration of fin whales through the Strait of Gibraltar. Marine Ecology Progress Series, 588: 215-228.

Gauffier P., et al. 2020. Wait your turn, North Atlantic fin whales share a common feeding ground sequentially. Mar. Environ. Res. 155: 104884.

Gavery M.R., Nichols K.M., Berejikian B.A., Tatara C.P., Goetz G.W., Dickey J.T., Van Doornik D.M., Swanson P. 2019. Temporal dynamics of DNA methylation patterns in response to rearing juvenile steelhead (*Oncorhynchus mykiss*) in a hatchery versus simulated stream environment. Genes, 10(5): 356.

Geiman T.M., Muegge K. 2010. DNA methylation in early development. Molecular Reproduction and Development: Incorporating Gamete Research, 77(2): 105-113.

Goto M., Kitakado T., Pastene L.A. 2020. A preliminary study of epigenetic estimation of age of the Antarctic minke whale *Balaenoptera bonaerensis*. Cetacean Population Studies, 2, 5–14.

Gwinner E. 1996. Circadian and Circannual programmes in avian migration. The Journal of Experimental Biology, 199: 39–48.

Häfker N.S., Meyer B., Last K.S., Pond D.W., Hüppe L., Teschke M. 2017. Circadian clock involvement in zooplankton diel vertical migration. Current Biology, 24; 27(14): 2194-2201.e3.

Hearn J., Pearson M., Blaxter M., Wilson P.J., Little T.J. 2019. Genome-wide methylation is modified by caloric restriction in Daphnia magna. BMC genomics, 20(1): 1-11.

Herrel A., Joly D., Danchin E. 2020. Epigenetics in ecology and evolution. Functional Ecology, 34:381–384.

Hida A., Kitamura S., Katayose Y., Kato M., Ono H., Kadotani H., Uchiyama M., Ebisawa T., Inoue Y., Kamei Y., Okawa M. 2014. Screening of clock gene polymorphisms demonstrates association of a *PER3* polymorphism with morningness–eveningness preference and circadian rhythm sleep disorder. Scientific reports, 4(1): 1-6.

IWC (International Whaling Commission). 2009. Report of the first intersessional RMP workshop on North Atlantic fin whales. The Journal of Cetacean Research and Management 11(Supplement): 425–452

Johnston R.A., Paxton K.L., Moore F.R., Wayne R.K., Smith T.B., 2016. Seasonal gene expression in a migratory songbird. Molecular Ecology, 25(22): 5680-5691.

Jones P., et al. 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics, 1;30(9): 1236 - 40.

Jover L. 1992. Morphometric differences between Icelandic and Spanish fin whales (*Balaenoptera physalus*). Report of the International Whaling Commission, 42: 747–750.

Jung M., Pfeifer G.P. 2015. Aging and DNA methylation. BMC biology, 13(1): 1-8.

Kemp C.M., Oliver W.T., Wheeler T.L., Chishti A.H., Koohmaraie M. 2013. The effects of *Capn1* gene inactivation on skeletal muscle growth, development, and atrophy, and the compensatory role of other proteolytic systems. Journal of animal science, 91(7): 3155-3167.

Klose R.J., Bird A.P. 2006. Genomic DNA methylation: The mark and its mediators. Trends in Biochemical Sciences, 31 (2): 89–97.

Ko C.H., Takahashi J.S. 2002. Molecular components of the mammalian circadian clock. Nature, 418: 935–941.

Kojima S., Matsumoto K., Hirose M., Shimada M., Nagano M., Shigeyoshi Y., Hoshino S.I., Ui-Tei K., Saigo K., Green C.B., Sakaki, Y. 2007. *LARK* activates posttranscriptional expression of an essential mammalian clock protein, *PERIOD1*. Proceedings of the National Academy of Sciences, 104(6): 1859-1864.

Kool J.T., Moilanen A., Treml E.A. 2013. Population connectivity: recent advances and new perspectives. Landscape Ecology, 28(2): 165-185.

Korashy H.M., El-Kadi, A.O. 2005. Regulatory mechanisms modulating the expression of cytochrome *P450 1A1* gene by heavy metals. Toxicological Sciences, 88(1): 39-51.

Krueger F., Andrews S.R. 2011. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics, 27(11): 1571-1572.

Langmead B., Salzberg S. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods, 9: 357–359.

Lea A.J., Altmann J., Alberts S.C., Tung J. 2016. Resource base influences genome-wide DNA methylation levels in wild baboons (*Papio cynocephalus*). Molecular Ecology, 25(8): 1681–1696.

Li S., et al. 2013. An optimized algorithm for detecting and annotating regional differential methylation. BMC Bioinformatics 14(Suppl. 5): S10.

Li X., Zhao H. 2020. Automated feature extraction from population wearable device data identified novel loci associated with sleep and circadian rhythms. PLoS genetics, 16(10): p.e1009089.

Liu C, Reppert S.M., 2000. GABA synchronizes clock cells within the suprachiasmatic circadian clock. Neuron, 25(1): 123-128.

Lourie S.A., Vincent A.C. 2004. Using biogeography to help set priorities in marine conservation. Conservation Biology, 18(4):1004-1020.

Ma M.A., Morrison E.H. Neuroanatomy, Nucleus Suprachiasmatic. [Updated 2021 Jul 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK546664/

Mancia A., Abelli L., Fossi M.C., Panti C. 2021. Skin distress associated with xenobiotics exposure: An epigenetic study in the Mediterranean fin whale (*Balaenoptera physalus*). Marine Genomics, 57: 100822.

Massicotte R., Whitelaw E., Angers B. 2011. DNA methylation: A source of random variation in natural populations. Epigenetics, 6(4): 421-427.

Markus M.A., Morris B.J. 2009. *RBM4*: a multifunctional RNA-binding protein. The international journal of biochemistry & cell biology, 41(4): 740-743.

McCarthy M.J. 2019. Missing a beat: assessment of circadian rhythm abnormalities in bipolar disorder in the genomic era. Psychiatric genetics, 29(2): 29-36.

McNew S.M., Beck D., Sadler-Riggleman I., Knutie S.A., Koop J.A.H., Clayton D.H., Skinner M.K. 2017. Epigenetic variation between urban and rural populations of Darwin's finches. BMC Evolutionary Biology, 17(1): 1–14.

Meissner A., et al. 2008. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature, 454(7205): 766-770

Meröndun J., Murray D.L., Shafer A.B.A. 2019. Genome-scale sampling suggests cryptic epigenetic structuring and insular divergence in Canada lynx. Molecular Ecology, 28: 3186–3196. DOI: 10.1111/mec.15131ature 454:766–770.

Metzger D.C., Schulte P.M. 2018. The DNA methylation landscape of stickleback reveals patterns of sex chromosome evolution and effects of environmental salinity. Genome biology and evolution, 10(3): 775-785.

Miyamoto-Mikami E., Kumaga H., Kikuchi N., Kamiya N., Miyamoto N., Fuku N. 2020. eQTL variants in *COL22A1* are associated with muscle injury in athletes. Physiological Genomics, 52(12): 588-589.

Molinaro, P. et al. 2013. Genetically modified mice as a strategy to unravel the role played by the Na+/Ca 2+ exchanger in brain ischemia and in spatial learning and memory deficits. Sodium calcium exchange: a growing spectrum of pathophysiological implications: 213-222.

Neph S., et al. 2012. BEDOPS: high-performance genomic feature operations. Bioinformatics, 28(14): 1919-1920.

Nilsen F.M., Parrott B.B., Bowden J.A., *et al*. 2016. Global DNA methylation loss associated with mercury contamination and aging in the American alligator (*Alligator mississippiensis*). Science of the Total Environment, 545: 389-397.

Palsbøll P.J., Berubé M., Aguilar A., Notarbartolo-Di-Sciara G., Nielsen R. 2004. Discerning between recurrent gene flow and recent divergence under a finite-site mutation model applied to North Atlantic and Mediterranean sea fin whale (*Balaenoptera physalus*) populations. Evolution, 58(3): 670–675.

Palsbøll P.J., Berube M., Allendorf F.W. 2007. Identification of management units using population genetic data. Trends in ecology & evolution, 22(1): 11-16.

Pampoulie C., Daníelsdóttir A.K. 2013. Review on the genetic stock structure of North Atlantic fin whales (*Balaenoptera physalus*): Past, present and future. IWC document SC/65a/RMP03, 1-8.

Pan Z.Z. 2012. Transcriptional control of *Gad2*. Transcription, 3(2): 68-72.

Pereira A., Harris D., Tyack P., Matias L. 2020. Fin whale acoustic presence and song characteristics in seas to the southwest of Portugal. The Journal of the Acoustical Society of America, 147(4): 2235-2249.

Philibert R., Dogan M., Beach S.R., Mills J.A., Long J.D. 2020. AHRR methylation predicts smoking status and smoking intensity in both saliva and blood DNA. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 183(1): 51-60.

Proietti Onori M., et al. 2018. The intellectual disability-associated CAMK2G p. Arg292Pro mutation acts as a pathogenic gain-of-function. Human mutation, 39(12): 2008-2024.

Ramos J.S.L., Delmore K.E., Liedvogel M., 2017. Candidate genes for migration do not distinguish migratory and non-migratory birds. Journal of Comparative Physiology A, 203(6): 383-397.

Reischl S., Kramer A. 2011. Kinases and phosphatases in the mammalian circadian clock. FEBS letters, 585(10): 1393-1399.

Reppert S., Weaver D. 2002. Coordination of circadian timing in mammals. Nature, 418: 935–941.

Rey O., Eizaguirre C., Angers B., Baltazar-Soares M., Sagonas K., Prunjer J.G., Blanchet S. 2020. Linking epigenetics and biological conservation: Towards a conservation epigenetics perspective. Functional Ecology, 34: 414– 427.

Robbins J. Dendanto D., Giard J., Panigada S., Sears R., Zanardelli M. 2007. Photo-ID studies of fin whales in the North Atlantic Ocean and the Mediterranean Sea. Report of the Scientific Committee of the International Whaling Commission SC/59/PF11, 1(4).

Ruegg K., Anderson E.C., Boone J., Pouls J., Smith T.B. 2014. A role for migration-linked genes and genomic islands in divergence of a songbird. Molecular Ecology, 23(19): 4757-4769.

Salem M., Nath J., Rexroad C.E., Killefer J., Yao, J. 2005. Identification and molecular characterization of the rainbow trout calpains (Capn1 and Capn2): their expression in muscle wasting during starvation. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 140(1): 63-71.

Sanpera C., Aguilar A. 1992. Modern whaling off the Iberian Peninsula during the 20th century. Report of the International Whaling Commission, 42: 723-730.

Sanpera C., González M., Jover L. 1996. Heavy metals in two populations of North Atlantic fin whales (*Balaenoptera physalus*). Environmental Pollution, 91(3): 299-307

Siegfried Z., Simon I. 2010. DNA methylation and gene expression. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 2(3): 362-371.

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(10): e76507.

Silva M.A., Borrell A., Prieto R., Gauffier P., Bérubé M., Palsbøl P.J., Colaço A. 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. Royal Society open Science, 6: 181800.

Su J., Yu Q., Yang J., Zheng N., Zhong J., Ji L., Li J., Chen X. 2021. The Association of polymorphisms in related circadian rhythm genes and clopidogrel resistance susceptibility. Basic & Clinical Pharmacology & Toxicology.

Supek F., Bošnjak M., Škunca N., Šmuc T. 2011. REVIGO Summarizes and visualizes long lists of gene ontology terms. PLOS ONE 6(7): e21800.

Taylor B.L., Martien K., Morin P. 2010. Identifying units to conserve using genetic data. Marine mammal ecology and conservation — a handbook of techniques. Oxford University Press, Oxford: 306-344.

Tokizane T., et al. 2005. Cytochrome P450 1B1 is overexpressed and regulated by hypomethylation in prostate cancer. Clinical cancer research, 11(16): 5793-5801.

Tollis M., et al. 2019. Return to the Sea, Get Huge, Beat Cancer: An Analysis of Cetacean Genomes Including an Assembly for the Humpback Whale (*Megaptera novaeangliae*). Molecular Biology and Evolution, 36(8): 1745-1763.

Vighi M., Borrell A., Aguilar A. 2016. Stable isotope analysis and fin whale population structure in the eastern North Atlantic. Marine Mammal Science, 32(2): 535–551.

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

Viola A.U., Archer S.N., James L.M., Groeger J.A., Lo J.C., Skene D.J., von Schantz M., Dijk D.J. 2007. *PER3* polymorphism predicts sleep structure and waking performance. Current biology, 17(7): 613-618.

Visser F., Hartman K.L., Pierce G.J., Valavanis V.D., Huisman J. 2011. Timing of migratory baleen whales at the Azores in relation to the North Atlantic spring bloom. Marine Ecology Progress Series, 440:.267-279.

Walters A.D., Schwartz M.K. 2020. Population genomics for the management of wild vertebrate populations. Population Genomics: Wildlife, p. 419.

Watanabe S., Shimada T., Nakamura S., Nishiyama N., Yamashita N., Tanabe S., Tatsukawa, R. 1989. Specific profile of liver microsomal cytochrome P-450 in dolphin and whales. Marine environmental research, 27(1): 51-65.

Wilson, J. Y., et al. 2005. Systemic effects of arctic pollutants in beluga whales indicated by CYP1A1 expression. Environmental health perspectives, 113(11), 1594-1599.

Yim H.S., Cho Y., Guang X. et al. 2014. Minke whale genome and aquatic adaptation in cetaceans. Nat Genet, 46: 88–92.

Zhou, H., Qu, Y., Wu, H., Liao, C., Zheng, J., Diao, X., Xue, Q. 2010. Molecular phylogenies and evolutionary behavior of AhR (aryl hydrocarbon receptor) pathway genes in aquatic animals: implications for the toxicology mechanism of some persistent organic pollutants (POPs). Chemosphere, 78(2), 193-205.

Table S1. Mapping performance when using as reference the minke whale genome.

Sample ID	Aligned Reads	% Aligned Reads	Total Cs	Methylated CpGs	Unmethylated CpGs
IC86008	16,964,842	52.78	183,513,604	15,640,078	10,660,629
IC86013	41,752,771	54.29	380,985,167	20,031,546	11,386,164
IC86016	15,622,224	48.38	183,099,767	15,998,996	15,722,810
IC86018	39,221,729	54.13	342,294,713	14,988,373	8,060,050
IC86027	16,733,758	52.08	188,120,939	17,060,014	14,022,517
IC86032	16,898,713	52.92	193,896,926	18,687,514	14,816,171
IC86042	15,909,856	38.42	150,747,164	7,913,936	6,043,781
IC86048	13,405,612	52.36	158,237,134	15,513,521	13,855,236
SP84002	20,802,574	47.84	186,135,302	8,742,978	6,555,180
SP84059	17,126,660	52.17	196,768,226	15,189,111	13,345,230
SP84062	11,263,145	53.04	127,691,712	10,135,640	8,987,528
SP84071	18,865,691	51.91	227,612,398	19,986,277	18,543,581
SP84073	27,140,268	50.69	343,317,981	32,476,353	30,324,307
SP84074	17,899,998	52.43	205,338,882	19,847,196	15,239,407
SP84083	17,911,587	48.09	169,051,626	10,031,594	6,426,030
SP84084	17,214,165	52.78	190,556,854	17,125,789	12,317,917
SP84087	18,729,502	53.43	193,120,819	15,122,773	10,979,648
SP84092	29,713,338	52.16	339,633,059	26,545,024	24,458,280
SP84093	13,758,884	53.69	146,680,785	11,924,858	8,979,070

Table S2. Mapping performance when using as reference the humpback whale genome.

Sample ID	Aligned Reads	% Aligned Reads	Total Cs	Methylated CpGs	Unmethylated CpGs
IC86008	18,566,379	57.77	201,621,219	17,906,487	11,660,411
IC86013	45,418,417	59.06	415,962,070	23,256,253	12,116,625
IC86016	17,109,115	52.98	2017,637	18,054,269	17,379,350
IC86018	42,579,859	58.77	372,699,395	17,418,302	8,490,185
IC86027	18,228,995	56.73	205,416,321	19,365,276	15,172,716
IC86032	18,464,515	57.82	212,377,073	21,118,585	16,053,636
IC86042	17,9624	43.38	170,210,821	9,618,462	6,838,485
IC86048	14,560,503	56.88	172,064,476	17,355,817	14,936,141
SP84002	22,676,512	52.15	203,581,448	10,175,585	7,016,849
SP84059	18,747,273	57.10	216,159,058	17,118,176	14,665,451
SP84062	12,286,403	57.86	139,784,313	11,526,037	9,803,425
SP84071	20,620,255	56.74	249,240,747	22,366,081	20,262,879
SP84073	29,697,928	55.47	377,160,545	36,584,719	33,299,600
SP84074	19,501,747	57.12	224,256,512	22,243,372	16,539,722
SP84083	19,558,830	52.51	184,985,183	11,509,738	6,852,308
SP84084	18,766,196	57.54	208,455,192	19,384,448	13,340,341
SP84087	20,331,202	58.00	210,473,435	17,284,994	11,852,211
SP84092	32,449,282	56.96	372,392,905	30,164,267	26,746,411
SP84093	154,063	58.55	160,669,704	13,677,089	9,827,180

Table S3. DMRs associated with minke whale genes. Meth diff. indicates the comparison between both populations, with positive values indicating increased methylation and negative values indicating decreased methylation in individuals from the Spanish population. CpGs indicates the total number of CpGs, and DMCs indicates the number of these CpGs presenting differential methylation between both populations. q-value indicates the significance of each DMR.

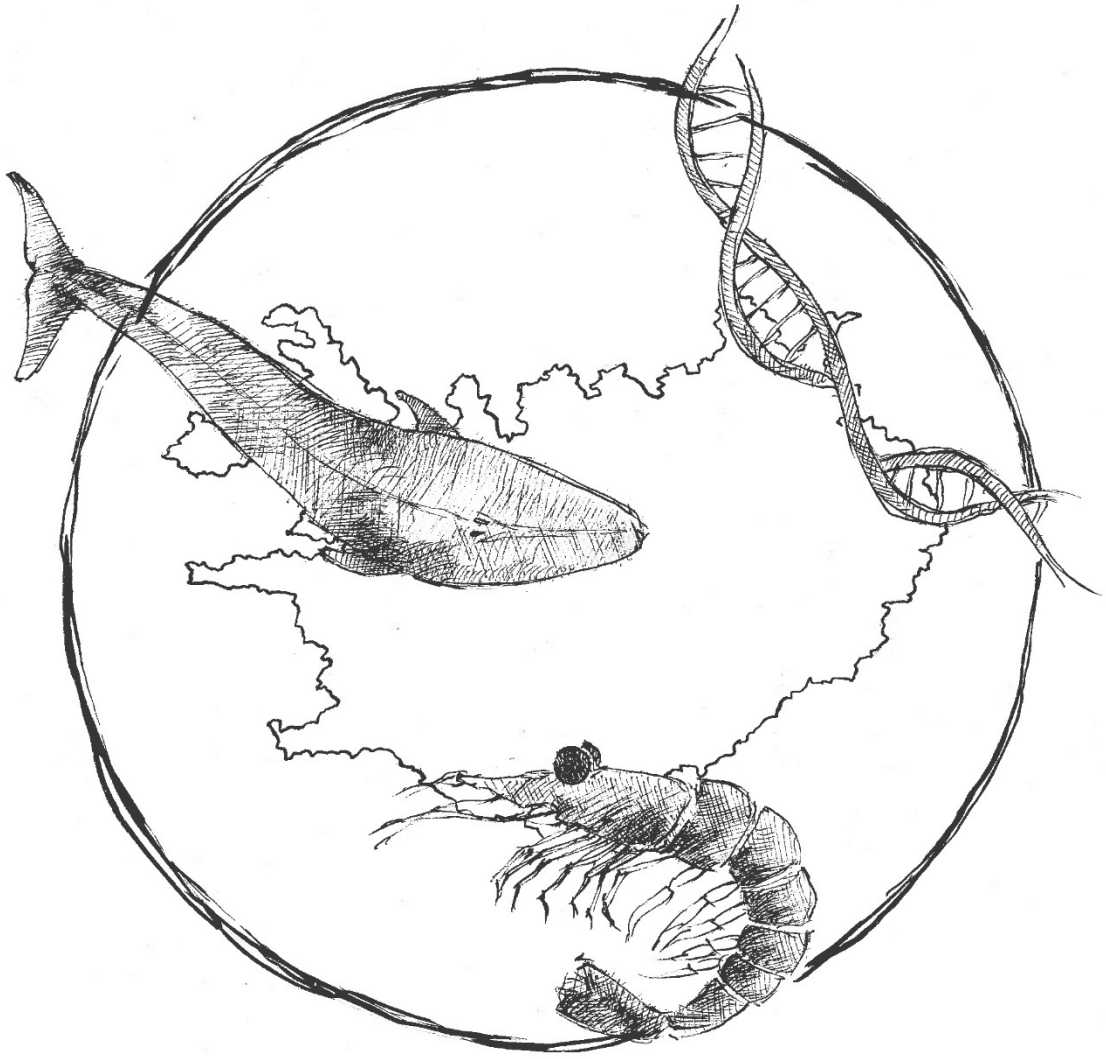
ID	Name	Meth diff	CpGs	DMCs	q-value
TIE1	Tyrosine Kinase With Immunoglobulin Like And EGF Like Domains 1	23,6	3	1	1,66E-26
ANKRD27	Ankyrin Repeat Domain 27	-21,6	3	1	2,19E-25
PTPRN2	Protein Tyrosine Phosphatase Receptor Type N2	-22,4	5	2	2,13E-21
CCDC27	Coiled-Coil Domain Containing 27	-21,4	3	1	2,42E-19
CAMK2G	Calcium/Calmodulin Dependent Protein Kinase II Gamma	-23,3	3	1	7,32E-09
MROH1	Maestro Heat Like Repeat Family Member 1	25,3	4	2	6,06E-16
CAMSAP2	Calmodulin Regulated Spectrin Associated Protein Family Member 2	23,4	3	1	9,55E-14
CNGB1	Cyclic Nucleotide Gated Channel Subunit Beta 1	25,6	3	1	7,21E-20
ADGRG1	Adhesion G Protein-Coupled Receptor G1	22,8	3	2	1,36E-28
PEBP4	Phosphatidylethanolamine Binding Protein 4	-34,7	3	3	6,94E-25
COL12A1	Collagen Type XII Alpha 1 Chain	-22,2	6	1	1,06E-40
ATP1A3	ATPase Na+/K+ Transporting Subunit Alpha 3	20,2	3	1	3,21E-18
SLC8A2	Solute Carrier Family 8 Member A2	23,1	3	1	4,15E-15
VAV1	Vav Guanine Nucleotide Exchange Factor 1	-26,8	3	2	7,53E-22
PER3	Period Circadian Regulator 3	-42,0	3	3	1,72E-41
COL16A1	Collagen Type XVI Alpha 1 Chain	-20,8	5	2	2,59E-15
CAPN1	Calpain 1	-22,8	3	1	1,28E-11
RBM4B	RNA Binding Motif Protein 4B	-28,9	5	4	2,61E-36
OSBPL5	Oxysterol Binding Protein Like 5	31,4	3	2	2,21E-26
KCNQ1	Potassium Voltage-Gated Channel Subfamily Q Member 1	26,2	3	1	3,51E-18
FAM193A	Family With Sequence Similarity 193 Member A	-28,7	3	1	3,96E-25
DBNDD1	Dysbindin Domain Containing 1	26,4	3	1	4,00E-21
COTL1	Coactosin Like F-Actin Binding Protein 1	20,1	7	2	4,79E-36
RARB	Retinoic Acid Receptor Beta	-23,1	3	1	6,88E-17
ZFAND2A	Zinc Finger AN1-Type Containing 2A	21,0	3	2	2,57E-23
HSPG2	Heparan Sulfate Proteoglycan 2	-25,6	4	1	1,18E-16
HOXB5	Homeobox B5	33,2	3	3	3,08E-23
HOXB6	Homeobox B6	37,3	5	5	5,59E-121

CISD3	CDGSH Iron Sulfur Domain 3	-22,2	4	2	6,14E-20
MLLT6	MLLT6, PHD Finger Containing	-22,2	4	2	6,14E-20
IKZF1	IKAROS Family Zinc Finger 1	-26,3	3	2	5,48E-17
FGFR1	Fibroblast Growth Factor Receptor 1	41,8	3	3	1,87E-48
LYPLAL1	Lysophospholipase Like 1	34,6	4	3	2,61E-51
IQSEC1	IQ Motif And Sec7 Domain ArfGEF 1	25,8	3	1	3,94E-39
HOXC6	Homeobox C6	-22,1	3	1	3,53E-23
PIP4K2C	Phosphatidylinositol-5-Phosphate 4-Kinase Type 2 Gamma	-28,2	4	3	1,65E-20
ANKRD17	Ankyrin Repeat Domain 17	21,4	3	2	3,20E-27
NAPSA	Napsin A Aspartic Peptidase	-27,9	3	2	2,24E-25
NPAS3	Neuronal PAS Domain Protein 3	24,3	3	2	1,02E-14
ZNF268	Zinc Finger Protein 268	28,9	3	2	4,44E-27
RBFOX2	RNA Binding Fox-1 Homolog 2	-25,5	4	1	6,16E-36
NSMF	NMDA Receptor Synaptonuclear Signaling And Neuronal Migration Factor	-24,4	3	2	2,29E-20
HEG1	Heart Development Protein With EGF Like Domains 1	-27,2	3	2	1,65E-28
NTM	Neurotrimin	24,5	3	1	2,95E-23
NTM	Neurotrimin	-27,7	3	1	1,77E-26
COL22A1	Collagen Type XXII Alpha 1 Chain	-21,7	3	1	1,54E-17
CPA6	Carboxypeptidase A6	23,5	4	2	7,85E-31
MSC	Musculin	-20,0	5	1	9,44E-35
CRISPLD1	Cysteine Rich Secretory Protein LCCL Domain Containing 1	21,2	3	1	1,25E-14
GNAS	GNAS Complex Locus	-21,2	4	1	1,19E-15
FAM210B	Family With Sequence Similarity 210 Member B	27,3	3	2	7,11E-25
ZMYND8	Zinc Finger MYND-Type Containing 8	24,0	4	3	2,03E-27
RPS6KA2	Ribosomal Protein S6 Kinase A2	29,7	3	2	2,13E-17
ATP5MF	ATP Synthase Membrane Subunit F	22,8	3	1	9,88E-17
CRTAC1	Cartilage Acidic Protein 1	-20,4	3	1	1,44E-11
CYP26C1	Cytochrome P450 Family 26 Subfamily C Member 1	-22,3	3	1	1,95E-33
PAX3	Paired Box 3	-33,8	4	4	9,53E-43
EXT1	Exostosin Glycosyltransferase 1	20,0	5	1	1,47E-27
ASAP1	ArfGAP With SH3 Domain, Ankyrin Repeat And PH Domain 1	20,5	6	1	3,34E-27
KHDRBS3	KH RNA Binding Domain Containing, Signal Transduction Associated 3	21,3	3	2	1,22E-24
RANGAP1	Ran GTPase Activating Protein 1	25,6	4	1	2,83E-24
PACSIN2	Protein Kinase C And Casein Kinase Substrate In Neurons 2	-26,4	6	2	9,10E-51
MPPED1	Metallophosphoesterase Domain Containing 1	-20,1	7	3	1,92E-68
BICDL1	BICD Family Like Cargo Adaptor 1	-22,5	3	2	1,90E-10
SLC15A4	Solute Carrier Family 15 Member 4	28,7	3	2	1,78E-35

FZD10	Frizzled Class Receptor 10	29,3	4	4	3,98E-50
GAD2	Glutamate Decarboxylase 2	-21,2	3	1	7,30E-29
TBX2	T-Box Transcription Factor 2	-24,1	3	2	1,86E-08
STK32B	Serine/Threonine Kinase 32B	-20,5	3	1	2,19E-21
AHRR	Aryl-Hydrocarbon Receptor Repressor	25,1	4	1	1,38E-35
NEDD4L	NEDD4 Like E3 Ubiquitin Protein Ligase	-23,8	3	1	2,24E-25
PRRT3	Proline Rich Transmembrane Protein 3	22,9	3	2	1,16E-20
TAMM41	TAM41 Mitochondrial Translocator Assembly And Maintenance Homolog	-24,7	4	2	1,51E-15
FGD5	FYVE, RhoGEF And PH Domain Containing 5	-20,4	3	1	2,39E-14
PALLD	Palladin, cytoskeletal associated protein	25,8	3	1	1,33E-15
DACT1	Dishevelled Binding Antagonist Of Beta Catenin 1	24,5	4	2	4,41E-34
SYNE2	Spectrin Repeat Containing Nuclear Envelope Protein 2	22,1	4	1	2,19E-23
PKHD1	PKHD1 Ciliary IPT Domain Containing Fibrocystin/Polyductin	22,4	7	3	8,79E-78
UCHL1	Ubiquitin C-Terminal Hydrolase L1	22,4	7	3	8,79E-78
ATF6B	Activating Transcription Factor 6 Beta	27,9	3	2	8,58E-30
FKBPL	FKBP Prolyl Isomerase Like	27,9	3	2	8,58E-30
PCDHGC4	Protocadherin Gamma Subfamily C, 4	27,3	3	2	4,28E-42
OLFM1	Olfactomedin 1	-21,2	3	2	6,30E-14
PLPP7	Phospholipid Phosphatase 7 (Inactive)	-26,2	3	1	8,49E-44
RALGPS1	Ral GEF With PH Domain And SH3 Binding Motif 1	-21,5	3	1	1,19E-15
SCG3	Secretogranin III	-23,8	3	2	1,43E-27
ASAP2	ArfGAP With SH3 Domain, Ankyrin Repeat And PH Domain 2	39,5	4	4	1,19E-61
SASH1	SAM And SH3 Domain Containing 1	21,9	5	4	8,22E-56
SHB	SH2 Domain Containing Adaptor Protein B	-27,7	3	1	4,06E-21
COL15A1	Collagen Type XV Alpha 1 Chain	20,1	3	1	8,24E-18
CAP2	Cyclase Associated Actin Cytoskeleton Regulatory Protein 2	20,2	4	1	2,12E-20
TBC1D8	TBC1 Domain Family Member 8	27,8	5	4	4,00E-65
MECOM	MDS1 And EVI1 Complex Locus	23,7	3	1	2,27E-14
KCTD1	Potassium Channel Tetramerization Domain Containing 1	-28,9	3	1	6,46E-25
HEXA	Hexosaminidase Subunit Alpha	24,1	3	1	2,74E-31
HMGN1	High Mobility Group Nucleosome Binding Domain 1	24,1	9	3	2,46E-90
TK2	Thymidine Kinase 2	21,7	3	1	1,79E-23

Table S4. Summary of our results for GO Biological Process ontology terms which were significantly enriched in our DMRs.

ID	p value	Count	NAME
GO:0051345	0,0074	2	positive regulation of hydrolase activity
GO:0065007	0,0033	18	biological regulation
GO:0097320	0,0185	1	plasma membrane tubulation
GO:0006351	0,0225	9	transcription, DNA-templated
GO:0019219	0,0017	11	regulation of nucleobase-containing compound metabolic process
GO:0044091	0,0277	1	membrane biogenesis
GO:0031175	0,0457	1	neuron projection development
GO:0019438	0,0446	9	aromatic compound biosynthetic process
GO:0018130	0,0473	9	heterocycle biosynthetic process
GO:0019222	0,0039	11	regulation of metabolic process
GO:0071709	0,0277	1	membrane assembly
GO:0031579	0,0277	1	membrane raft organization
GO:0050794	0,0092	16	regulation of cellular process
GO:0032774	0,0227	9	RNA biosynthetic process
GO:0051336	0,0177	2	regulation of hydrolase activity
GO:0034654	0,0405	9	nucleobase-containing compound biosynthetic process



DISCUSIÓN

DISCUSIÓN

Los resultados de los trabajos incluidos en esta tesis cubren distintas lagunas de conocimiento, relacionadas con la dieta, migración y estructura poblacional del rorcual común de Islandia. En esta sección se procederá a resumir, integrar y discutir los principales resultados obtenidos para cada una de estas cuestiones, englobándolas en dos bloques generales. En el primer bloque se tratará la dieta del rorcual común y el solapamiento de ésta con los otros rorcuales que habitan en las mismas aguas, mientras que en el segundo bloque se tratarán las migraciones y la estructura poblacional del rorcual común de Islandia.

Bloque 1: Dieta e interacciones interespecíficas

Entre otras aplicaciones, los isótopos estables han sido ampliamente utilizados para inferir la dieta (ver por ejemplo Hopkins III and Ferguson 2012) y el estado nutricional (ver por ejemplo Polischuk et al. 2001) en una gran diversidad de especies. En esta tesis, se han analizado los isótopos estables de nitrógeno, carbono y azufre en dos tejidos con propiedades metabólicas marcadamente distintas.

En el **Capítulo 1**, se analizaron muestras de piel de las especies de misticetos que ocupan las aguas islandesas en verano para inferir su dieta durante la época de alimentación, así como las posibles competencias por los recursos entre las ballenas que acuden en verano a las aguas islandesas. Esta discusión general se ha centrado en los resultados directamente relacionados con el rorcual común, pero pueden encontrarse resultados adicionales en el artículo incluido en el Capítulo 1.

En los **Capítulos 2 y 3**, se analizaron barbas de distintos individuos de rorcual común. Las barbas de las ballenas crecen de manera continuada, pero una vez el tejido ha sido depositado en la encía, éste queda biológicamente inerte y su composición no se altera con el tiempo. Este depósito progresivo permite que se genere un registro secuencial de los valores isotópicos de los animales, reflejando los cambios que ocurren en la dieta o en la ubicación geográfica durante una ventana temporal de alrededor de dos años.

Dieta en la zona de alimentación e interacciones con otros rorcuales en Islandia

Las ratios de los isótopos estables de nitrógeno y carbono analizados en piel de rorcual común de Islandia indicaron que esta especie se alimenta casi exclusivamente de krill.

Aunque el krill está considerado la presa principal del rorcual común del Atlántico Norte (Aguilar and García-Vernet 2018), en algunas regiones geográficas se ha visto que una parte significativa de la dieta está compuesta de pescado, incluyendo especies como el capelán o el arenque (Jonsgård 1966, Kawamura 1980, Aguilar and García-Vernet 2018). Esta aparente contradicción seguramente esté explicada por un cambio de dieta a lo largo de la estación, con un incremento de consumo de pescado al acercarse la época otoñal (MFRI, datos no publicados). En el **Capítulo 1**, la mayoría de las muestras de piel de rorcual común fueron obtenidas a mediados de verano, época en la que parece que se especializa en el consumo de krill, mientras que el consumo de pescado queda relegado a un porcentaje muy marginal.

Otro factor importante que merece la pena ser comentado es la posible competencia interespecífica entre el rorcual común y otras especies de rorcuales que se alimentan en aguas islandesas. Los resultados de los isótopos estables de nitrógeno y carbono mostraron una segregación significativa entre el rorcual común y la ballena jorobada, el rorcual aliblanco y el rorcual boreal. A pesar de que en todas las especies mencionadas el krill fue la presa principal, la ballena jorobada y el rorcual aliblanco también presentaron un consumo de pescado destacable, en consonancia con lo descrito en otras regiones del Atlántico Norte (Clapham 2018, Pike et al. 2019, Víkingsson et al. 2014). Por otra parte, el rorcual boreal se situó en el nivel trófico más bajo, reflejando un consumo considerable de copépodos (Sigurjónsson 1995). Por tanto, aunque todas las especies de rorcuales dependen sustancialmente del krill, es probable que la competencia interespecífica se vea mitigada por la presencia de pescado y de copépodos en la dieta de las ballenas jorobadas, los rorcuales aliblancos y los rorcuales boreales.

Sin embargo, en el caso de la ballena azul, los resultados de los isótopos de nitrógeno y carbono mostraron una alimentación basada casi exclusivamente en krill, como ocurre en el caso del rorcual común. Aunque no existen estudios previos de la dieta de las ballenas azules en Islandia, nuestros resultados son concordantes con las observaciones realizadas en otras regiones del Atlántico Norte (Sears and Perrin 2018), y serían una explicación plausible al alto porcentaje de asociaciones descritas entre individuos de rorcual común y ballena azul (Aguilar and García-Vernet 2018). No obstante, este claro solapamiento de la dieta, reflejado en las ratios isotópicas del nitrógeno y el carbono, apunta a una posible competencia entre estas dos especies.

Aunque las presas que componen la dieta de las ballenas azules y los rorcuales comunes sean altamente coincidentes, existen otros métodos para evitar la competición

interespecífica. Por ejemplo, la segregación espacial (ocupación de áreas distintas) o la segregación temporal (ocupación secuencial de las aguas islandesas) permiten reducir la presión ejercida sobre un mismo recurso ecológico y disminuir la competencia (Clapham and Brownell 1996). En esta línea, las ratios de los isótopos estables de azufre nos proporcionan información relevante sobre el grado de segregación espacial, ya que los valores de $\delta^{34}S$ se reducen de manera considerable en zonas costeras que reciben aportes de agua dulce (Nehlich 2015). Las ratios de los isótopos de azufre de rorcual común y ballena azul no presentaron diferencias significativas, probablemente reflejando un comportamiento mayoritariamente oceánico; no obstante, y a pesar de la falta de significación, las diferencias fueron mayores que las observadas para las ratios de nitrógeno y carbono, sugiriendo un cierto grado de segregación espacial. Basándonos en los valores de $\delta^{34}S$ del **Capítulo 1**, sería posible que los rorcuales comunes fueran algo más costeros que las ballenas azules.

Sin embargo, es necesario destacar que en Islandia se ha observado que las ballenas azules tienen un comportamiento más costero, ubicándose mayoritariamente en la plataforma continental, mientras que el rorcual común parece ser más oceánico (Pike et al. 2009, Pike et al. 2019), a diferencia de lo observado en otras regiones del Atlántico Norte, en las cuales las ballenas azules también se localizan en alta mar (Lesage et al. 2017). Una potencial explicación sería que los valores de $\delta^{34}S$ de las ballenas azules estén reflejando parcialmente la señal isotópica previa a su llegada a Islandia; esta hipótesis está reforzada en el hecho de que, a nivel secuencial, las ballenas azules son de las últimas especies en llegar a Islandia (Sigurjónsson and Víkingsson 1992, 1997). Sin embargo, por el momento se trata tan solo de una hipótesis, ya que actualmente se desconoce la tasa de recambio de los valores de $\delta^{34}S$ en piel de ballena.

Comportamiento alimentario en las zonas de invernada

Fuera de las zonas de alimentación, existe poca información disponible sobre el comportamiento alimentario del rorcual común. Tradicionalmente, se ha considerado que la alimentación invernal es escasa o inexistente, ya que las hipotéticas zonas de reproducción son oligotróficas y ofrecen pocas oportunidades de alimentación (Aguilar and García-Vernet 2018). Sin embargo, algunos estudios recientes han constatado la existencia de alimentación ocasional durante los movimientos migratorios (Silva et al. 2013), por lo cual el escenario planteado inicialmente podría ser más complejo de lo que se creía en un principio.

Como puede verse en los **Capítulos 2** y **3**, los análisis isotópicos de nitrógeno, carbono y azufre realizados a lo largo de la longitud de barbas de los distintos individuos mostraron oscilaciones, reflejando las migraciones estacionales descritas en el rorcual común (Aguilar and García Vernet 2018). Además, los resultados del **Capítulo 2** indicaron una correlación consistente entre los valores de $\delta^{15}N$ y $\delta^{34}S$. Aunque esta correlación no se observó al analizar la piel (ver **Capítulo 1**), sí se había descrito anteriormente en barbas de ballenas de Groenlandia (Matthews and Ferguson 2015). Estos resultados podrían estar indicando que los valores de $\delta^{34}S$ no reflejan solamente cambios en la localización espacial de las ballenas, sino también cambios en la dieta, ya sea relacionados con un cambio de nivel trófico o con un ayuno estacional.

La comparación entre los resultados obtenidos en el **Capítulo 1** (análisis en pieles) y en el **Capítulo 2** (análisis en barbas) nos permite descartar con bastante seguridad la opción de que los valores de $\delta^{34}S$ estén reflejando un cambio de nivel trófico. En general, los valores de $\delta^{34}S$ son bastante homogéneos entre distintos tejidos (Arneson and MacAvoy 2005, Webb et al. 2017), y la discriminación entre dieta y consumidor está considerada negligible (Krajcarz et al. 2021). Respaldando esta hipótesis, en el **Capítulo 1** puede observarse que algunas especies de ballenas de Islandia con distinto nivel trófico, reflejado en sus valores de $\delta^{15}N$ en piel, presentan valores muy similares de $\delta^{34}S$.

Los resultados obtenidos en el **Capítulo 2** muestran un incremento de los valores de $\delta^{15}N$ y $\delta^{34}S$ en las zonas de la barba que fueron sintetizadas en invierno, época en la cual podría estarse produciendo un posible ayuno. Durante periodos de elevado estrés nutricional, los animales utilizan como fuente de nitrógeno sus propias reservas proteicas, lo cual parece producir un incremento de los valores de $\delta^{15}N$ (Hobson et al. 1993, Lee et al. 2012). Aunque en el caso del azufre esta relación no está tan clara, también se ha apuntado que los periodos de ayuno o de bajo consumo de proteínas podrían producir un incremento de los valores $\delta^{34}S$ (Richards et al. 2003). Teniendo en cuenta que las barbas están principalmente formadas de queratina, cuyo aminoácido principal es la cisteína (Wang et al. 2016), y que la cisteína puede sintetizarse a partir de la metionina y la serina (Finkelstein 1990), podría ser que la contribución de azufre endógeno estuviera produciendo un enriquecimiento del ^{34}S (Richards et al. 2003).

Aunque esta hipótesis es concordante con los resultados presentados en el **Capítulo 2**, las oscilaciones observadas siguen el patrón opuesto al descrito por Matthews and Ferguson (2015): mientras que en los rorcuales comunes de Islandia se observa un aumento de los valores de $\delta^{15}N$ y $\delta^{34}S$ en invierno, en las ballenas de Groenlandia se produce una disminución de los valores de $\delta^{15}N$ y $\delta^{34}S$. Así mismo, en rorcuales

comunes de España también se ha observado un descenso de la señal de $\delta^{15}N$ durante periodos invernales, sugiriendo que en esta especie podría prevenirse el efecto del ayuno a través de depredación ocasional y adaptaciones metabólicas (Aguilar et al. 2014). Independientemente del motivo, lo que resulta evidente es que, si la relación entre los valores de $\delta^{15}N$ y $\delta^{34}S$ se debe a procesos metabólicos consecuencia del ayuno, la direccionalidad de las oscilaciones debería ser la misma en todos los estudios.

La última hipótesis plausible, contemplada por Matthews and Ferguson (2015), sería una variación coincidente de los valores de $\delta^{15}N$ y $\delta^{34}S$: mientras que los valores de $\delta^{34}S$ estarían reflejando principalmente cambios en la localización espacial, los valores de $\delta^{15}N$ estarían reflejando tanto la dieta como la localización geográfica. En el caso de los valores azufre, a nivel general se considera que los valores del océano son bastante homogéneos, situándose alrededor de 21‰ (Rees et al. 1978). No obstante, existen algunos factores que pueden afectar la señal isotópica del azufre. Por ejemplo, las emisiones de las dorsales oceánicas tienen valores de $\delta^{34}S$ que oscilan entre 3-13‰, mientras que las emisiones volcánicas oscilan entre -10 y 10‰ (Strauss 2004). Teniendo en cuenta que Islandia está situada en una zona volcánicamente activa (Einarsson 1991), estas emisiones podrían justificar los valores bajos de $\delta^{34}S$ en los segmentos de las barbas sintetizados en verano. Esta hipótesis se ve también respaldada por los resultados obtenidos en el **Capítulo 1**. Los valores de $\delta^{34}S$ de piel de rorcuales comunes de Islandia presentaron una señal empobrecida en ^{34}S respecto a los rorcuales comunes del mar Mediterráneo (Borrell et al. 2021): 18.8‰ ± 0.4 vs 19.3‰ ± 0.4, respectivamente. Estas diferencias, que a pesar de ser pequeñas resultan significativas, soportarían la hipótesis de que las aguas islandesas presentan valores más bajos de $\delta^{34}S$.

Por otra parte, la escasa magnitud de variación total que se observa en las oscilaciones del $\delta^{34}S$ respecto a las del $\delta^{15}N$ podría deberse al mismo motivo. Mientras que las oscilaciones del $\delta^{34}S$ estarían reflejando pequeñas variaciones en un océano bastante uniforme, las oscilaciones del $\delta^{15}N$ estarían reflejando tanto variaciones geográficas, probablemente ocasionadas por la asociación de los rorcuales a eventos de afloramientos (Mompeán et al. 2013, Rita 2021), como posibles cambios en la dieta. De hecho, los datos aquí presentados respaldan estudios previos que sugieren que los rorcuales comunes aumentan el consumo ocasional de pescado durante el invierno (Christensen et al. 1992, Rita 2021), lo cual se reflejaría en el aumento de los valores de $\delta^{15}N$ observados durante la época invernal.

En resumen, los resultados de esta tesis muestran que, durante los primeros meses de estancia en Islandia, el rorcual común se alimenta principalmente de krill, siendo el

consumo de pescado muy anecdótico. Así mismo, se observa un solapamiento de los nichos isotópicos del rorcual común y la ballena azul, debido a que ambas especies consumen casi exclusivamente krill. Esta competencia interespecífica podría verse mitigada por una cierta segregación espacial o temporal, reflejada principalmente en los valores de $\delta^{34}S$. Además, las oscilaciones isotópicas detectadas en barbas respaldan movimientos migratorios estacionales, y aunque no puede descartarse la existencia de un ayuno, los valores de $\delta^{34}S$ en las barbas parecen reflejar cambios en la ubicación geográfica de los individuos, y no cambios relacionados con la dieta. Por otra parte, el incremento en los valores de $\delta^{15}N$ durante la época de invierno podría indicar tanto cambios geográficos como un incremento del nivel trófico.

Bloque 2: Migraciones y estructura poblacional

En los artículos incluidos en esta tesis se utilizaron tanto análisis de isótopos estables, concretamente del nitrógeno (**Capítulo 3**) como análisis de la metilación del ADN (**Capítulos 4** y **5**) para tratar de resolver el comportamiento migratorio y la diferenciación poblacional del rorcual común de Islandia.

Como se presenta en el **Capítulo 3**, los resultados de los análisis isotópicos del nitrógeno, realizados a lo largo de la longitud de las barbas de 24 individuos, mostraron una alta variabilidad en el patrón de las oscilaciones. Una primera hipótesis sería que la variabilidad observada, tanto en la magnitud como en la amplitud de los ciclos, pueda deberse a diferencias en el tamaño de las barbas muestreadas. Según esta hipótesis, la diversidad que se encuentra en el tamaño de las barbas de un mismo animal sería consecuencia directa de tasas de crecimiento diferenciales. No obstante, en **el Capítulo 2** se descartó que el tamaño de las barbas, así como su posición relativa en la boca del rorcual, estuviera afectando al patrón de las oscilaciones. En ese capítulo, se determinó que la diferencia que se observa en el tamaño de las barbas de un mismo animal se debe a diferentes tasas de erosión según la posición relativa en la boca del rorcual.

Por tanto, esta variabilidad interindividual podría estar reflejando una dispersión de los individuos en aguas abiertas durante la época de invierno, un comportamiento que podría ser generalizado en las especies del género *Balaenoptera* (Mackintosh 1966, Payne and Webb 1971, Whitehead and Rendell 2015; Aguilar and García-Vernet 2018). Esta dispersión invernal proporcionaría mayores posibilidades de alimentarse de forma oportunista durante las migraciones y en las zonas reproductivas (Silva et al. 2013, Silva

et al. 2019), y explicaría también el aumento de los valores de $\delta^{15}N$ que se observa en los fragmentos de barba sintetizados en invierno (ver Bloque 1 de la Discusión).

El artículo incluido en el **Capítulo 3** también presentó otro resultado interesante: a pesar de la alta variabilidad observada, cuatro pares de individuos exhibieron patrones de oscilaciones muy similares, en algunos casos prácticamente idénticos. Teniendo en cuenta que los fragmentos de barbas analizados corresponderían aproximadamente a un registro temporal de 2 años, estos resultados sugieren la existencia de asociaciones interindividuales a largo plazo, algo novedoso ya que hasta la fecha no se han reportado asociaciones de este tipo en el género *Balaenoptera*. Una primera hipótesis planteada fue que estas similitudes estuvieran reflejando una potencial transmisión vertical de las estrategias migratorias (Whitehead and Rendell 2015). Sin embargo, los análisis genéticos descartaron relaciones de parentesco cercanas entre los individuos con patrones isotópicos idénticos.

El rorcual común, al igual que el resto de balaenoptéridos, está considerada una especie no gregaria, aunque es frecuente avistarlos en parejas. Además, en las zonas de alimentación es usual encontrar agrupaciones de decenas de individuos (Víkingsson et al. 2009, Joiris et al. 2014, Aguilar and García-Vernet 2018). Aunque los grupos numerosos son efímeros, es posible que los grupos pequeños de 2 – 3 individuos presenten una mayor estabilidad. De hecho, en la ballena jorobada se han reportado asociaciones interindividuales estables a lo largo de los años mientras están en las zonas de alimentación, generalmente entre parejas de individuos no emparentados (Weinrich 1991, Ramp et al. 2010, Ziegesar et al. 2021).

En el caso del rorcual común, resulta intrigante cómo podría combinarse una dispersión invernal con una estrategia de asociación en pequeños grupos estables a lo largo del tiempo. El rorcual común, al igual que las ballenas azules, produce pulsos de baja frecuencia que pueden propagarse largas distancias en el océano (Payne and Webb 1971). Aunque se considera que su uso principal es atraer a las hembras a zonas con presas (Croll et al. 2002, Romagosa et al. 2021), también podría ser que estos sonidos estuvieran facilitando una coordinación entre individuos durante las migraciones, como parece ocurrir en ballenas azules (Oestreich et al. 2020). Así pues, esta capacidad acústica facilitaría los movimientos grupales entre individuos dispersos en un área relativamente grande (Stern and Friedlaender 2018).

Finalmente es posible que, independientemente de las potenciales asociaciones entre individuos, algunas estrategias migratorias sean especialmente óptimas. Así pues, incluso bajo un contexto de dispersión invernal, podría ser que hubiera algunas

estrategias compartidas por un número considerable de individuos que darían lugar a patrones isotópicos con ciertas similitudes, como puede verse en los resultados del **Capítulo 3**. Por tanto, la compartición de estrategias migratorias similares daría también lugar a patrones isotópicos parecidos, sin la necesidad de que los individuos estuviesen migrando juntos a nivel espacial.

Los resultados expuestos en el **Capítulo 3** presentan un marco complejo. Los rorcuales comunes de Islandia parecen dispersarse en invierno, ya sea a nivel individual o en pequeños grupos, conformados mayoritariamente por dos individuos. Aunque es posible que existan estrategias migratorias especialmente óptimas que sean compartidas por múltiples individuos, resulta difícil inferir una estructura clara dentro de la población de rorcual común de Islandia. Estos resultados llevan inevitablemente a plantearse qué grado de conectividad puede existir con las otras poblaciones de rorcual común que también habitan en el Atlántico Norte.

Como se ha comentado anteriormente, la IWC determinó 7 stocks, basados principalmente en datos recopilados en las zonas de alimentación (IWC 2009). No obstante, la mayoría de los estudios genéticos, realizados tanto con marcadores nucleares como mitocondriales, han encontrado baja diferenciación genética entre stocks, sugiriendo un elevado grado de flujo genético entre los individuos que conforman las agrupaciones en las zonas alimentación (Palsboll et al. 2004, Berubé et al. 2006, Pampoulie and Danielsdottir 2013). Para tratar de arrojar algo de luz, en los **Capítulos 4** y **5** se realizaron análisis epigenéticos en individuos de dos stocks del Atlántico Norte: Islandia y España. Aunque entre ambos grupos no parece existir una diferenciación genética considerable (Berubé et al. 1998), el aislamiento de ambas poblaciones ha sido sugerido a raíz de análisis realizados con técnicas no moleculares (Víkingsson and Gunnlaugsson 2005).

En los estudios presentados en ambos capítulos se encontraron diferencias significativas entre poblaciones. Sin embargo, cabe destacar que el objetivo principal del estudio del **Capítulo 4** era evaluar el uso de los niveles de metilación en posiciones CpG de 3 genes concretos (*TET2*, *CDKN2A*, *GRIA2*) para inferir la edad de los rorcuales, dato fundamental para entender algunos parámetros poblacionales. Los resultados mostraron que, aunque algunas posiciones CpG variaban con la edad, esta variación no era lo suficientemente acusada como para permitir predicciones precisas, como si se había logrado en ballenas jorobadas (Polanowski et al. 2014), pero obteniendo resultados similares a los descritos en rorcuales aliblancos (Goto et al. 2020, Tanabe et al. 2020). No obstante, se observó que en una de las posiciones CpG del gen

TET2, los niveles de metilación eran significativamente distintos entre ambas poblaciones.

Queriendo ahondar en estos resultados, en el **Capítulo 5** se procedió a ampliar los análisis previos, secuenciando los metilomas de individuos pertenecientes a los stocks de Islandia y de España. Aunque las técnicas de secuenciación actuales permiten testear diferencias a nivel de CpG individuales, en general las dianas reguladas por cambios en la metilación suelen agruparse en regiones cortas. Por tanto, trabajar con regiones en lugar de con posiciones discretas permite aumentar la robustez de los análisis y, al mismo tiempo, dotar de sentido biológico los resultados (Gaspar and Hart 2017, Lent et al. 2018). Se identificaron 215 regiones diferencialmente metiladas, de las cuales 96 estabas asociadas a genes anotados en el genoma de rorcual aliblanco, y un 10% de éstos estuvieron asociados a genes involucrados en el reloj circadiano. El reloj circadiano participa en la regulación de los ritmos fisiológicos (Ko and Takashi 2006), y permite la anticipación a una gran variedad de estímulos asociados con fluctuaciones diarias. Así mismo, se considera que el reloj circadiano es un elemento clave regulador de los procesos migratorios (Coppack 2008, Häfker et al. 2017).

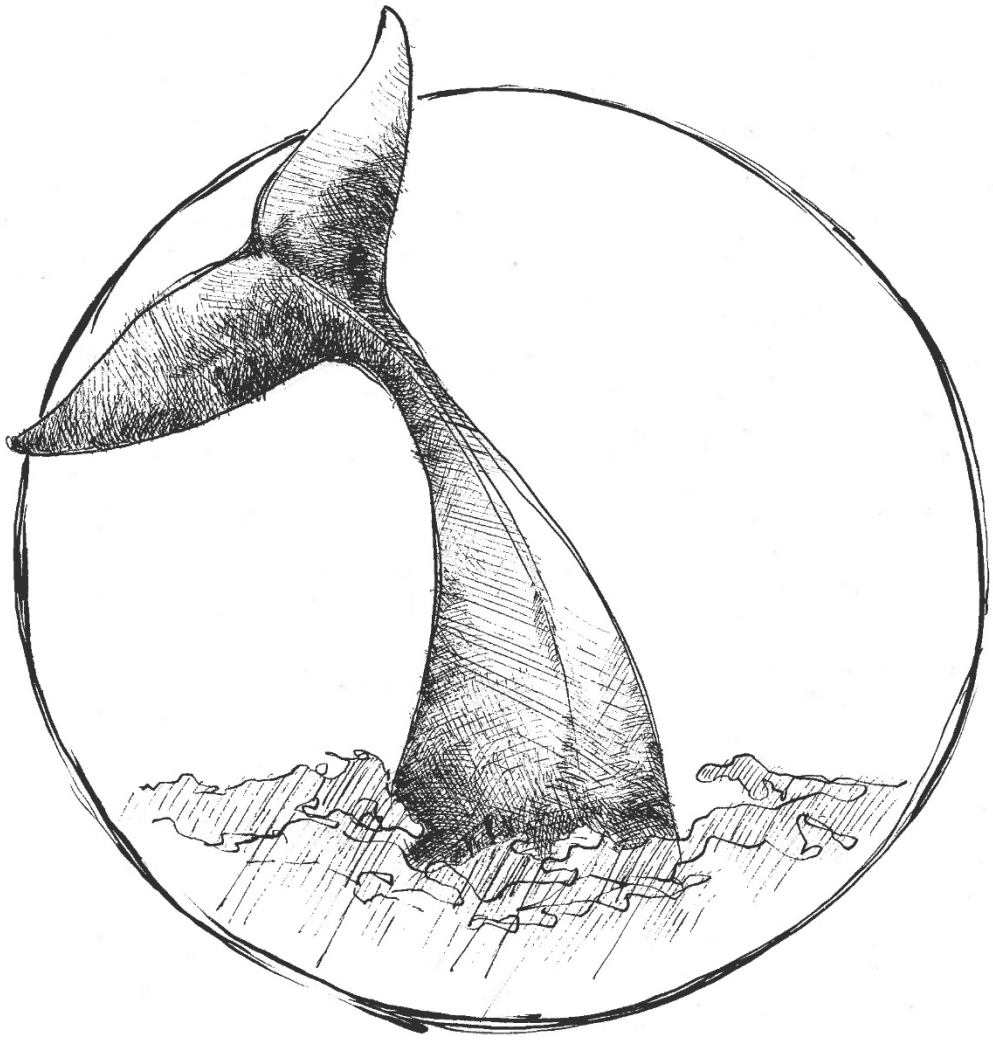
Estas diferencias entre poblaciones, que se encuentran detalladas en el artículo que se incluye en el **Capítulo 5**, pueden ser el reflejo de patrones migratorios diferenciales entre ambas poblaciones. El stock de España parece estar compuesto principalmente de individuos pertenecientes a una unidad reproductora no relacionada con el resto de los rorcuales comunes del Atlántico Norte, mientras que la composición del stock de Islandia sigue bajo debate (IWC 2009). Las estadísticas de capturas balleneras de la estación de Caneliñas, situada en la costa de Galicia, muestran un pico de abundancia en julio y agosto (Sanpera and Aguilar 1992), concordante con el uso de esta región como zona de alimentación (IWC 2009), aunque también se llegaron a capturar rorcuales comunes durante el otoño, invierno y la primavera (Sanpera and Aguilar 1992). Estos datos sugieren que, aunque el grueso de la población parece migrar a lo largo del otoño, es posible que algunos individuos no efectúen migraciones y/o que ocurra una ocupación secuencial por parte de rorcuales comunes de otros stocks. El destino invernal del grueso del stock de España no está claro, pero en el sur de Portugal se detecta un pico de abundancia de patrones acústicos asociados a rorcuales Atlánticos entre noviembre y enero (Pereira et al. 2020). Así mismo, parece que una parte de estos podrían hacer incursiones al mar Mediterráneo (Castellote et al. 2012, Gauffier et al. 2018, Pereira et al. 2020), mientras que otra podría moverse hacia la costa oeste africana (Vighi et al. 2016).

Por otra parte, el stock de rorcual común de Islandia parece estar compuesto principalmente de individuos con zonas reproductoras situadas en el Atlántico Central, incluyendo las Azores (IWC 2009, Silva et al. 2013). Sin embargo, algunos estudios recientes sugieren una ocupación secuencial de las aguas situadas al noroeste de España. De esta manera, se ha descrito que algunos rorcuales que se encuentran en las Azores en primavera, y que en verano parecen migrar hacia Groenlandia e Islandia, parecen haberse alimentado en las costas españolas antes de moverse hacia el Atlántico Central y las Azores (Silva et al. 2019, Gauffier et al. 2020).

En cualquier caso, incluso teniendo en cuenta una posible coincidencia en las costas españolas, es evidente que los rorcuales pertenecientes a ambos stocks estarán efectuando migraciones muy distintas, con los individuos de Islandia viajando mayores distancias que los individuos de España. Así mismo, es probable que las señales ambientales que los rorcuales pueden usar como indicadores de inicio de migración en las zonas de alimentación, tales como el fotoperiodo (Bani Assadi and Fraser 2021), temperatura (Burnside et al. 2021) o el seguimiento de presas (Visser et al. 2011, Abrahms et al. 2019), presenten enormes diferencias entre las costas españolas e islandesas. Teniendo en cuenta que los rorcuales comunes parecen ser bastante consistentes en cuanto a las zonas de alimentación que visitan (ver por ejemplo Robbins et al. 2007), parece razonable esperar cierto grado de adaptación a las condiciones ambientales específicas de su zona de alimentación preferencial. Por tanto, ya que es probable que exista un cierto grado de flujo genético entre ambos stocks (Berubé et al. 1998, Berubé et al. 2006), los cambios en los niveles de metilación, inducidos a raíz de diferencias ambientales, podrían estar proporcionando heterogeneidad adaptativa a estas dos poblaciones aparentemente homogéneas a nivel genético (Flores et al. 2013, Meröndun et al. 2019).

En resumen, los resultados de esta tesis apuntan a que los rorcuales comunes de Islandia tienden a dispersarse durante el invierno. Sin embargo, algunas parejas de individuos exhiben patrones isotópicos prácticamente idénticos durante al menos dos años, lo cual sugiere que se puedan producir asociaciones a largo plazo entre parejas de individuos sin parentesco. También parece probable que existan estrategias migratorias óptimas que son compartidas por diversos individuos. Por último, y a pesar de la diversidad de patrones migratorios que presentan los rorcuales del stock de Islandia, los análisis epigenéticos respaldan diferencias adaptativas entre los individuos que se alimentan en las costas islandesas y los individuos que se alimentan en las costas españolas. En concreto, se encontró un número considerable de genes relacionados con los ritmos circadianos, lo cual respalda que estos dos stocks estarían

realizando migraciones diferenciales que requieren de adaptaciones específicas, independientemente del grado de conectividad existente entre ambas poblaciones.



CONCLUSIONES

CONCLUSIONES

- Durante los primeros meses de verano, el rorcual común de la población islandesa se alimenta casi de forma exclusiva de krill. El consumo de pescado es marginal, aunque podría ser que incrementara durante los meses de otoño e invierno.

- En Islandia, el solapamiento entre los nichos isotópicos del rorcual común y la ballena azul es elevado, debido a que ambas especies tienen una composición de dieta muy similar. Es probable que este solapamiento se vea mitigado por un cierto grado de segregación temporal y/o espacial, reflejada en los isótopos de azufre.

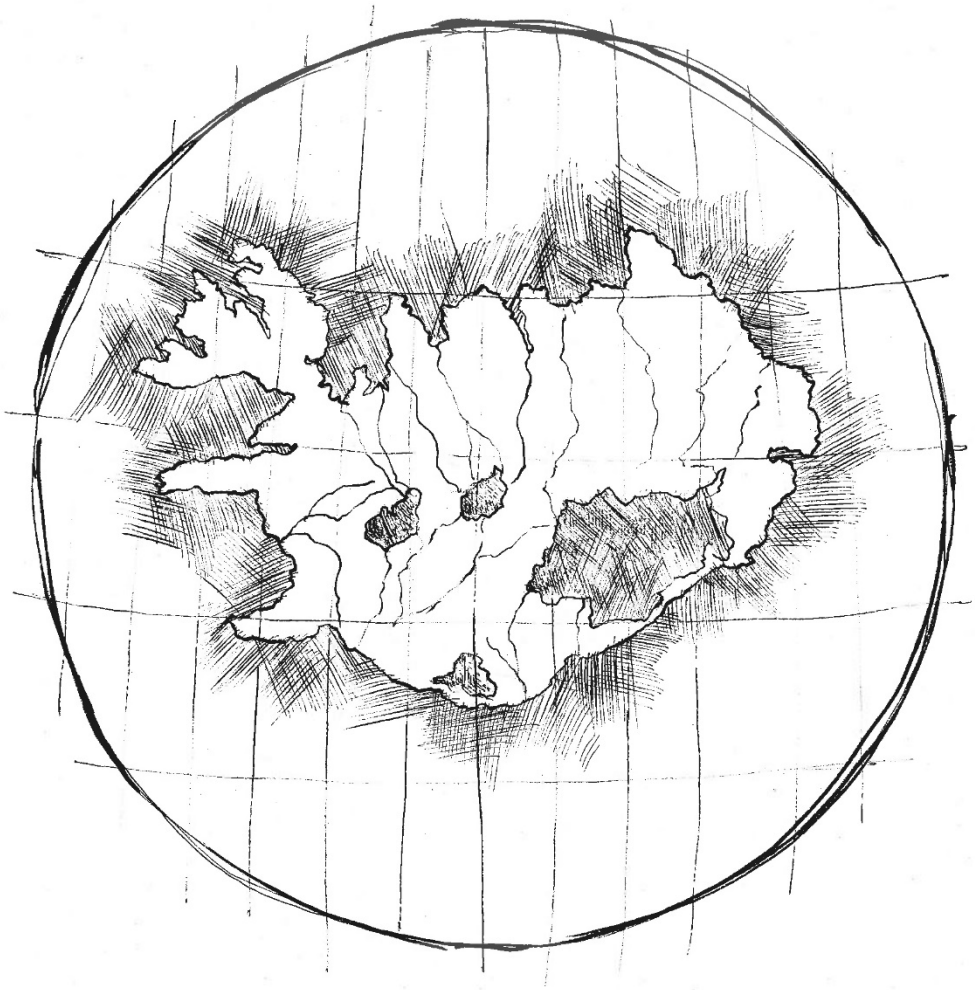
- La diferencia que se observa en el tamaño de las barbas de un mismo animal no se debe a tasas de crecimiento distintas, sino a tasas de erosión diferentes según la posición relativa de la barba en la boca del rorcual. Este resultado permite garantizar que los datos obtenidos a partir de barbas de diversos tamaños son comparables entre sí.

- Las razones isotópicas de azufre en las barbas demuestran ser una herramienta útil para detectar movimientos a escala oceánica. Aunque se detecta una correlación consistente entre los valores del $\delta^{34}S$ y el $\delta^{15}N$, los resultados obtenidos apuntan a que los isótopos de azufre reflejan movimientos entre zonas con distintos valores base de $\delta^{34}S$. Por otra parte, los resultados de los isótopos de nitrógeno apuntan a un posible incremento de nivel trófico de los rorcuales comunes durante la época invernal.

- Las razones isotópicas de nitrógeno en las barbas reflejan una alta variabilidad de patrones migratorios, lo cual apunta a que los rorcuales comunes siguen una estrategia de dispersión durante el invierno. Sin embargo, algunos individuos presentan patrones isotópicos idénticos, sugiriendo posibles asociaciones estables a lo largo de los años entre algunos animales no emparentados. También parece probable que existan algunas estrategias migratorias especialmente óptimas, que serían compartidas por diversos individuos

- Los niveles de metilación en regiones de los genes *TET2*, *CDKN2A* y *GRIA2*, no son útiles para establecer un modelo fiable capaz de inferir la edad de los rorcuales comunes. Aun así, se observó una relación lineal entre el incremento de metilación y la edad de los animales.

- Las poblaciones de rorcual común de Islandia y de España presentan regiones metiladas diferencialmente a nivel de epigenoma. Un considerable número de los genes presentes en estas regiones estaban relacionados con el ciclo circadiano u otras características fenotípicas asociadas a la migración, reflejando posibles adaptaciones específicas en cada una de las poblaciones



BIBLIOGRAFÍA

BIBLIOGRAFÍA

Abrahms B., Hazen E.L., Aikens E.O., Savoca M.S., Goldbogen J.A., Bograd S.J., Jacox M.G., Irvine L.M., Palacios D.M., Mate, B.R. 2019. Memory and resource tracking drive blue whale migrations. Proceedings of the National Academy of Sciences, 116(12): 5582-5587. DOI: 10.1073/pnas.1819031116

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

Aguilar A., Lockyer C.H. 1987. Growth, physical maturity, and mortality of fin whales (*Balaenoptera physalus*) inhabiting the temperate waters of the northeast Atlantic. Canadian Journal of Zoology, 65: 253-264

Aguilar A., Giménez J., Gómez–Campos E., Cardona L., Borrell A. 2014. δ15N Value does not reflect fasting in mysticetes. PLoS ONE 9(3): e92288. DOI: 10.1371/journal.pone.0092288

Aguilar A., García-Vernet R. 2018. Fin whale. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals (Third edition): 368-371 Ahuja N., Issa J-P.J. 2000. Aging, methylation and cancer. Histology and Histopathology, 15: 835-842. DOI: 10.14670/HH-15.835

Archer F.I., Morin P.A., Hancock-Hanser B.L., Robertson K.M., Leslie M.S., Berubé M., Panigada S., Taylor B.L. 2013. Mitogenomic phylogenetics of fin whales (*Balaenoptera physalus* spp.): Genetic evidence for revision of subspecies. PLoS ONE 8(5): e63396. DOI: 10.1371/journal.pone.0063396

Arregui M., Borrell A., Víkingsson G., Ólafsdóttir D., Aguilar A. 2018 Stable isotope analysis of fecal material provides insight into the diet of fin whales. Marine Mammal Science, 34: 1059–1069. DOI: 10.1111/mms.12504

Árnason U., Gullberg A., Janke A. 2004. Mitogenomic analyses provide new insights into cetacean origin and evolution. Gene 333: 27–34. DOI: 10.1016/j.gene.2004.02.010

Árnason U., Lammers F., Kumar V., Nilsson M.A., Janke A. 2018. Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow. Science Advances, 4: eaap9873. DOI: 10.1126/sciadv.aap9873.

Arneson L.S., MacAvoy S.E. 2005. Carbon, nitrogen, and sulfur diet–tissue discrimination in mouse tissues. Canadian Journal of Zoology, 83(7): 989–995. DOI: 10.1139/z05-083

Baerwald M.R., Meek M.H., Stephens M.R., Nagarajan R.P., Goodbla A.M., Tomalty K.M.H., Thorgaard G.H., May B., Nichols K.M. 2016. Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. Molecular Ecology, 25(8): 1785-1800. DOI: 10.1111/mec.13231

Baker C.S., Steel D., Calambokidis J., et al. 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Marine Ecology Progress Series, 494: 291–306. DOI: 10.3354/meps10508.

Bani Assadi S., Fraser K.C. 2021 Experimental manipulation of photoperiod influences migration timing in a wild, long-distance migratory songbird. Proceedings of Royal Society B, 288: 20211474. DOI: 10.1098/rspb.2021.1474

Bannister J.L. 2018. Baleen whales (mysticeti). In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), Encyclopedia of Marine Mammals (Third Edition): 62-69.

Barendse J., Best P.B., Carvalho I., Pomilla C. 2013. Mother Knows Best: Occurrence and Associations of Resighted Humpback Whales Suggest Maternally Derived Fidelity to a Southern Hemisphere Coastal Feeding Ground. PLoS ONE, 8(12): e81238. DOI: 10.1371/journal.pone.0081238

Berbel-Filho W.M., Berry N., Rodríguez-Barreto D., Rodrigues Teixeira D., Garcia de Leaniz C., Consuegra S. 2020. Environmental enrichment induces intergenerational behavioural and epigenetic effects on fish. Molecular Ecology, 29: 2288–2299. DOI: 10.1111/mec.15481

Berubé M., Aguilar A., Dendanto D., Larsen F., Notarbartolo di Sciara G., Sears R., Sigurjónsson J., Urban-R J., Palsboll P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. Molecular Ecology, 7: 585–599.

Berubé M., et al. 2006. High rates of gene flow among geographic locations in North Atlantic fin whales (*Balaenoptera physalus*). IWC/ SC/58/PFI6.

Bettridge S., Baker C.S., Barlow J., et al. 2015. Status review of the humpback whale (Megaptera novaeangliae) under the endangered species act 2015. NOAA Technical Memorandum NMFS SWFSC-540: 240

Boecklen W.J., Yarnes C.T., Cook B.A., James A.C. 2011. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics, 42: 411–40. DOI: 10.1146/annurev-ecolsys-102209-144726

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

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. Physiological and Biochemical Zoology 89(1):41–50. DOI: 10.1086/684632.

Borrell A., Gazo M., Aguilar A., Raga J.A., Degollada E., Gozalbes P., García-Vernet R. 2021. Niche partitioning amongst northwestern Mediterranean cetaceans using stable isotopes. Progress in Oceanography 193: 102559. DOI: 10.1016/j.pocean.2021.102559.

Bors E.K, Baker C.S., Wade P.R., O'Neill K.B., Shelden K.E.W., Thompson M.J., Fei Z., Jarman S., Horvath S. 2021. An epigenetic clock to estimate the age of living beluga whales. Evolutionary Applications, 14: 1263–1273. DOI: 10.1111/eva.13195

Boyle P., Clement K., Gu H., Smith Z.D., Ziller M., Fostel J.L., Holmes L., Meldrim J., Kelley F., Gnirke A., Meissner A. 2012. Gel-free multiplexed reduced representation bisulfite sequencing for large-scale DNA methylation profiling. Genome Biology, 13: R92. DOI: 10.1186/gb-2012-13-10-r92

Burnside R.J., Salliss D., Collar N.J., Dolman P.M. 2021. Birds use individually consistent temperature cues to time their migration departure. Proceedings of the National Academy of Sciences, 118(28): e2026378118

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 (5): e0177880. DOI: 10.1371/journal.pone.0177880

Cabrera A.A., et al. 2019. Fin whale (*Balaenoptera physalus*) mitogenomics: A cautionary tale of defining sub-species from mitochondrial sequence monophyly. Molecular Phylogenetics and Evolution, 135: 86–97. DOI: 10.1016/j.ympev.2019.02.003

Castellote M., Clark C.W., Lammers M.O. 2012. Fin whale (*Balaenoptera physalus*) population identity in the western Mediterranean Sea. Marine Mammal Science, 28(2): 325-344. DOI: 10.1111/j.1748-7692.2011.00491.x

Caut S., Angulo E., Courchamp F. 2008. Caution on isotopic model use for analyses of consumer diet. Canadian Journal of Zoology, 86(5): 438-445. DOI: 10.1139/Z08-012

Caut S., Laran S., Garcia-Hartmann E., Das K. 2010. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). The Journal of Experimental Biology 214, 538-545. DOI: 10.1242/jeb.045104

Christensen I., Haug T., Oien N. 1992. A review of feeding and reproduction in large baleen whales (Mysticeti) and sperm whales *Physeter macrocephalus* in Norwegian and adjacent waters. Fauna Norvegica Series A, 13: 39–48.

Clapham P.J., Brownell R.L. 1996. The potential for interspecific competition in baleen whales. Reports of the International Whaling Commission, 46: 361–367.

Clapham P.J. 2018. Humpback whale. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), Encyclopedia of Marine Mammals (Third Edition): 489-492

Clapham P.J., Baker C. S. 2018. Whaling, Modern. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), Encyclopedia of Marine Mammals (Third Edition): 1070 – 1074.

Cooke J.G. 2018a. *Balaenoptera musculus* (errata version published in 2019). The IUCN Red List of Threatened Species 2018: e.T2477A156923585. DOI: 10.2305/IUCN.UK.2018-2.RLTS.T2477A156923585.enCooke, J.G. 2018b. *Balaenoptera physalus*. The IUCN Red List of Threatened Species 2018: e.T2478A50349982. DOI: 10.2305/IUCN.UK.2018-2.RLTS.T2478A50349982.en

Coppack T., Becker S.F., Becker P.J.J. 2008. Circadian flight schedules in night-migrating birds caught on migration. Biology Letters, 4: 619–622 DOI: 10.1098/rsbl.2008.0388

Coplen T.B. 2011. Guidelines and recommended terms for expression of stable isotope-ratio and gas-ratio measurement results. Rapid Communications in Mass Spectrometry, 25: 2538–2560. DOI: 10.1002/rcm.5129

Croll D.A., Clark C.W., Acevedo A., Tershy B., Flores S., Gedamke J., Urban J. 2002. Only male fin whales sing loud songs. Nature, 417: 809-809. DOI: 10.1038/417809a

Darst R.P., Pardo C.E., Ai L., Brown K.D., Kladde M.P. 2012. Bisulfite Sequencing of DNA. Current Protocols in Molecular Biology, 7.9.1-7.9.17. DOI: 10.1002/0471142727.mb0709s91

De Paoli-Iseppi R., Deagle B.E., McMahon C.R., Hindell M.A., Dickinson J.L., Jarman S.N. 2017. Measuring animal age with DNA methylation: from humans to wild animals. Frontiers in Genetics, 8: 106. DOI: 10.3389/fgene.2017.00106

De Paoli-Iseppi R., Deagle B.E., Polanowski A.M., McMahon C.R., Dickinson J.L., Hindell M.A., Jarman S.N. 2018. Age estimation in a long-lived seabird (*Ardenna tenuirostris*) using DNA methylation-based biomarkers. Molecular Ecology Resources, 19: 411–425. DOI: 10.1111/1755-0998.12981

Deaton A.M., Bird A. 2011. CpG islands and the regulation of transcription. Genes & Development, 25: 1010–1022. DOI: 10.1101/gad.2037511

Dhingra R., Nwanaji-Enwerem J.C., Samet M., Ward-Caviness C.K.2018. DNA methylation age—environmental influences, health impacts, and its role in environmental epidemiology. Current Environmental Health Reports, 5:317–327. DOI: 10.1007/s40572-018-0203-2

Donovan G.P. 1991. A review of IWC stock boundaries. Reports of the International Whaling Commission, Special Issue Series, 13: 39-68.

Edwards E.F., Hall C., Moore T.J., Sheredy C., Redfern J.V. 2015. Global distribution of fin whales *Balaenoptera physalus* in the post-whaling era (1980–2012). Mammal Review, 45(4): 197–214. DOI: 10.1111/mam.12048

Einarsson P. 1991. Earthquakes and present-day tectonism in Iceland. Tectonophysics 189: 261-279. DOI: 10.1016/0040-1951(91)90501-I

Feil R., Fraga M.F. 2012. Epigenetics and the environment: emerging patterns and implications. Nature Reviews Genetics, 13: 97–109. DOI: 10.1038/nrg3142

Finkelstein J.D. 1990. Methionine metabolism in mammals. The Journal of nutritional biochemistry, 1(5): 228–37. DOI: 10.1016/0955-2863(90)90070-2

Flores K.B., Wolschin F., Amdam G.V. 2013. The Role of Methylation of DNA in Environmental Adaptation. Integrative and Comparative Biology, 53(2): 359–372. DOI: 10.1093/icb/ict019

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

Gavrilchuk K., Lesage V., Ramp C., Sears R., Bérubé M., Bearhop S., Beauplet, G. 2014. Trophic niche partitioning among sympatric baleen whale species following the collapse of groundfish stocks in the Northwest Atlantic. Marine Ecology Progress Series, 497: 285–301. DOI: 10.3354/meps10578

Gaspar J.M., Hart R.P. 2017. DMRfinder: efficiently identifying differentially methylated regions from MethylC-seq data. BMC bioinformatics, 18(1): 1-8. DOI: 10.1186/s12859-017-1909-0

Gauffier P., Verborgh P., Giménez J., Esteban R., Sierra J.M.S., de Stephanis R. 2018. Contemporary migration of fin whales through the Strait of Gibraltar. Marine Ecology Progress Series, 588: 215-228. DOI : 10.3354/meps12449

Gauffier P., Borrell A., Silva M.A., et al. 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

Giménez J., Ramírez F., Almunia J., Forero M.G., de Stephanis R. 2016. From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*). Journal of Experimental Marine Biology and Ecology, 475: 54–61. DOI: 10.1016/j.jembe.2015.11.001

Goel N., Karir P., Garg V.K. 2017. Role of DNA methylation in human age prediction. Mechanisms of Ageing and Development, 166: 33–41. DOI: 10.1016/j.mad.2017.08.012

Goto M., Kitakado T., Pastene L.A. 2020. A preliminary study of epigenetic estimation of age of the Antarctic minke whale *Balaenoptera bonaerensis*. Cetacean Population Studies, 2, 5–14. DOI: 10.34331/cpops.2.1_5

Grönniger E., Weber B., Heil O., Peters N., Stäb F., Wenck H., Korn B., Winnefeld M., Lyko F. 2010. Aging and chronic sun exposure cause distinct epigenetic changes in human skin. Plos Genetics, 6(5): e1000971. DOI: 10.1371/journal.pgen.1000971

Gunnlaugsson T., Vikingssson G.A. 2014. Winter occurrence of whales in waters around Iceland. International Whaling Commission, SC/65b/RMP06. RMP06, 1–6. (accessed 1 May 2020) https ://iwc.int/home.

Häfker N.S., Meyer B., Last K.S., Pond D.W., Hüppe L., Teschke M. 2017. Circadian clock involvement in zooplankton diel vertical migration. Current Biology, 24; 27(14): 2194-2201.e3. DOI: 10.1016/j.cub.2017.06.025.

Hannum G., et al. 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. Molecular Cell 49: 359–367. 10.1016/j.molcel.2012.10.016

Herman D.P., Yitalo G.M., Robbins J., Straley J.M., Gabriele C.M., Clapham P.J., Boyer R.H., Tilbury K L., Pearce R.W., Krahn M.M. 2009. Age determination of humpback whales Megaptera novaeangliae through blubber fatty acid compositions of biopsy samples. Marine Ecology Progress Series, 392: 277–293. DOI: 10.3354/meps08249

Hobson K.A. 1987. Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diets. Canadian Journal of Zoology, 65(5): 1210-1213. DOI: 10.1139/z87-187

Hobson K.A., Alisauskas R.T., Clark R.G. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. Condor, 95: 388–394

Hobson K.A., Piatt J.F., Pitocchellij J. 1994. Using stable isotopes to determine seabird trophic relationships. Journal of Animal Ecology, 63: 786-798. DOI: 10.2307/5256

Hoefs J. 2018. Stable Isotope Geochemistry (Eight edition). Springer International Publishing AG. DOI: 10.1007/978-3-319-78527-1

Hopkins III J.B., Ferguson J.M. 2012. Estimating the diets of animals using stable isotopes and a comprehensive Bayesian mixing model. PloS one, 7(1): e28478. DOI: 10.1371/journal.pone.0028478.s002

Horvath S. 2013. DNA methylation age of human tissues and cell types. Genome Biology, 14: 3156. DOI: 10.1186/gb-2013-14-10-r115

Horvath S., Raj K. 2018. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nature Reviews Genetics, 19: 371 – 384. DOI: 10.1038/s41576-018-0004-3

Hyung-Soon Y., et al. 2014. Minke whale genome and aquatic adaptation in cetaceans. Nature Genetics, 46: 88–92. DOI: 10.1038/ng.2835.

Ito H., Udono T., Hirata S., Inoue-Murayama M. 2018. Estimation of chimpanzee age based on DNA methylation. Scientific Reports, 8: 9998. DOI:10.1038/s41598-018-28318-9.

IWC (International Whaling Commission). 2009. Report of the first intersessional RMP workshop on North Atlantic fin whales. The Journal of Cetacean Research and Management 11(Supplement): 425–452

Jacobsen, S. C., et al. 2012. Effects of short-term high-fat overfeeding on genome-wide DNA methylation in the skeletal muscle of healthy young men. Diabetologia, 55(12): 3341–3349. DOI: 10.1007/s00125-012-2717-8

Joiris C.R., Falck E., D'Hert D., Jungblut S., Boos K. 2014. An important late summer aggregation of fin whales *Balaenoptera physalus*, little auks *Alle alle* and Brünnich's guillemots *Uria lomvia* in the eastern Greenland Sea and Fram Strait: influence of hydrographic structures. Polar Biology, 37: 1645–1657. DOI: 10.1007/s00300-014-1551-5

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

Jover L. 1992. Morphometric differences between Icelandic and Spanish fin whales (*Balaenoptera physalus*). Report of the International Whaling Commission, 42: 747–750.

Kawamura A. 1980. A review of food of balaenopterid whales. Scientific Report of the Whales Research Institute, 32: 155-197.

Kelly J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology, 78: 1–27

Klose R.B., Bird A.P. 2006. Genomic DNA methylation: the mark and its mediators. Trends in Biochemical Sciences, 31(2): 89-97. DOI: 10.1016/j.tibs.2005.12.008

Ko C.H., Takahashi J.S. 2002. Molecular components of the mammalian circadian clock. Nature, 418: 935–941. DOI: 10.1038/nature00965

Kota S.K., Feil R. 2010. Epigenetic transitions in germ cell development and meiosis. Developmental Cell, 19: 675-686. DOI: 10.1016/j.devcel.2010.10.009

Krajcarz M.T., Krajcarz M., Drucker D.G., Bocherens H. 2019. Prey-to-fox isotopic enrichment of 34S in bone collagen: Implications for paleoecological studies. Rapid Communications on Mass Spectrometry 33: 1311 – 1317. DOI: 10.1002/rcm.8471

Lea A.J., Altmann J., Alberts S.C., Tung J. 2016. Resource base influences genome-wide DNA methylation levels inwild baboons (*Papio cynocephalus*). Molecular Ecology, 25(8): 1681–1696. DOI: 10.1111/mec.13436

Lee T.N., Buck C.L., Barnes B.M., O'Brien D.M. 2012. A test of alternative models for increased tissue nitrogen isotope ratios during fasting in hibernating arctic ground squirrels. Journal of Experimental Biology, 215(19): 3354-61. DOI: 10.1242/jeb.068528

Lent S., Xu H., Wang L., Wang Z., Sarnowski C., Hivert M.F., Dupuis, J. 2018. Comparison of novel and existing methods for detecting differentially methylated regions. BMC genetics, 19(1): 27-31. DOI: 10.1186/s12863-018-0637-4

Lesage V., Gavrilchuk K., Andrews R.D., Sears R. 2017. Foraging areas, migratory movements and winter destinations of blue whales from the western North Atlantic. Endangered Species Res. 34, 27–43. DOI: 10.3354/esr00838

Levine M.E., et al. 2018. An epigenetic biomarker of aging for lifespan and healthspan. Aging, 10(4): 573-591. DOI: 10.18632/aging.101414

Lockyer C.H., Brown S.G. 1981. The migration of whales. In: Aidley DJ, editor. Animal Migration. Cambridge, Cambridge University Press: 105–137

Lockyer, C. 1982. Preliminary investigation of some anatomical characters of fin whale ear plugs collected from different regions of the NE Atlantic. Report of the International Whaling Commission, 32: 101-103.

Lockyer C.H. 1984. Age determination by means of the earplug in baleen whales. Report of the International Whaling Commission, 34: 692–696

Lott C.A., Meehan T.D., Heath J.A. 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: Influence of marine prey base. Oecologia, 134(4): 505–510. DOI: 10.1007/s00442-002-1153-8

Lydersen C., Vacquié-Garcia J., 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. Scientific Reports 10:16966. DOI: 10.1038/s41598-020-73996-z.

MacAvoy S.E., Bacalan V., Kazantseva M., Rhodes J., Kim K. 2015. Sulfur isotopes show importance of freshwater primary production for Florida manatees. Marine Mammal Science 31(2): 720–725. DOI: 10.1111/mms.12166

Mackintosh N.A. 1966. The distribution of southern blue and fin whales. In Whales, Dolphins, and Porpoises, Norris, K. S. Univ. of Calif. Press. Berkeley, Ed: 125-142.

Marx F.G., Fordyce R.E. 2015. Baleen boom and bust: a synthesis of mysticete phylogeny, diversity and disparity. Royal Society open science, 2: 140434. DOI: 10.1098/rsos.140434

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. Marine Ecology Progress Series 522: 269–286.

McCutchan J.H.Jr., Lewis W. M., Kendall C., McGrath C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos, 102: 378–390. DOI: 10.1034/j.1600-0706.2003.12098.x

McGowen M.R., Spaulding M., Gatesy J. 2009. Divergence date estimation and a comprehensive molecular tree of extant cetaceans. Molecular Phylogenetics and Evolution 53: 891–906. DOI: 10.1016/j.ympev.2009.08.018

McGowen M.R., Tsagkogeorga G., Alvarez-Carretero S., Dos reis M., Struebig M, Deaville R., Jepson P.D., Jarman S., Polanowski A., Morin P.A., Rossiter S.J. 2020. Phylogenomic Resolution of the Cetacean Tree of Life Using Target Sequence Capture. Systematic Biology, 69(3): 479–501. DOI: 10.1093/sysbio/syz068

McGuinness, D., et al. 2012. Socio-economic status is associated with epigenetic differences in the pSoBid cohort. International Journal of Epidemiology, 41(1): 151–160. DOI: 10.1093/ije/dyr215

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

McNew S.M., Beck D., Sadler-Riggleman I., Knutie S.A., Koop J.A.H., Clayton D.H., Skinner M.K. 2017. Epigenetic variation between urban and rural populations of Darwin's finches. BMC Evolutionary Biology, 17: 183. DOI 10.1186/s12862-017-1025-9

Merlin C., Liedvogel M. 2019. The genetics and epigenetics of animal migration and orientation: birds, butterflies and beyond. Journal of Experimental Biology, 222: jeb191890. DOI:10.1242/jeb.191890

Meröndun J., Murray D.L., Shafer A.B.A. 2019. Genome-scale sampling suggests cryptic epigenetic structuring and insular divergence in Canada lynx. Molecular Ecology, 28: 3186–3196. DOI: 10.1111/mec.15131

Mizroch S.A., Rice D.W., Breiwick J.M. 1984. The Fin Whale, *Balaenoptera physalus*. Marine Fisheries Review, 46: 20–24

Mompeán C., Bode A., Benítez-Barrios V.M., Domínguez-Yanes J.F., Escánez J., Fraile-Nuez E. 2013. Spatial patterns of plankton biomass and stable isotopes reflect the influence of the nitrogen-fixer *Trichodesmium* along the subtropical North Atlantic. Journal of plankton research, 35(3): 513-25. DOI: 10.1093/plankt/fbt011

Nehlich O. 2015. The application of sulphur isotope analyses in archaeological research: A review. Earth Science Reviews, 142: 1–17. DOI: 10.1016/j.earscirev.2014.12.002.

Niño-Torres C.A., Gallo-Reynoso J.P., Galván-Magaña F., Escobar-Briones E., Macko S.A. 2006. Isotopic analysis of δ13C, δ15N, and δ34S "a feeding tale" in teeth of the long beaked common dolphin, *Delphinus capensis*. Marine Mammal Science 22(4): 831–846. DOI: 10.1111/j.1748-7692.2006.00065.x

Olova N., Krueger F., Andrews S., Oxley D., Berrens R.V., Branco M.R., Reik W. 2018. Comparison of whole-genome bisulfite sequencing library preparation strategies identifies sources of biases affecting DNA methylation data. Genome Biology, 19: 33. DOI: 10.1186/s13059-018-1408-2.

Oestreich W.K., Fahlbusch J.A., Cade D.E., Calambokidis J., Margolina T., Joseph J., Friedlaender A.S., McKenna M.F., Stimpert A.K., Southall B.L., Goldbogen J.A. 2020. Animal-borne metrics enable acoustic detection of blue whale migration. Current Biology, 30(23): 4773-4779. DOI: 10.1016/j.cub.2020.08.105

Palsboll P.J., Berubé M., Aguilar A., Notarbartolo-Di-Sciara G., Nielsen R. 2004. Discerning between recurrent gene flow and recent divergence under a finite-site mutation model applied to North Atlantic and Mediterranean sea fin whale (*Balaenoptera physalus*) populations. Evolution, 58(3): 670–675. DOI: 10.1554/02-529.

Pálsson J., Astthorsson O.S., Valdimarsson H., 2012. Hydrographic variability in Icelandic waters during recent decades and related changes in distribution of some fish species. ICES Journal of Marine Science, 69: 816–825. DOI: 10.1093/icesjms/fss027

Pampoulie C., Daníelsdóttir A.K. 2013. Review on the genetic stock structure of North Atlantic fin whales (*Balaenoptera physalus*): Past, present and future. IWC document SC/65a/RMP03, 1-8.

Pampoulie C., Benónísdóttir S., Skaug H.J., Elvarsson B. Þ., Víkingsson G.A. 2013. Genetic relatedness of North Atlantic fin whale *Balaenoptera physalus* in Icelandic waters. IWC/SC/65a/RMP01

Payne R., Webb D. 1971. Orientation by means of long range acoustic signaling in baleen whales. Annals of the New York Academy of Sciences, 188: 110–141. DOI: 10.1111/j.1749-6632.1971.tb13093.x

Pereira A., Harris D., Tyack P., Matias L. 2020. Fin whale acoustic presence and song characteristics in seas to the southwest of Portugal. The Journal of the Acoustical Society of America, 147(4): 2235-2249. DOI: 10.1121/10.0001066

Peterson BJ. Fry B. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics. 18: 293–320.

Pike D.G., Gunnlaugsson T., Víkingsson G.A. 2002. Estimates of humpback whale (*Megaptera novaeangliae*) abundance in the North Atlantic, from NASS-95 shipboard survey data. Paper SC/54/H10 presented to the IWC Scientific Committee, 13pp

Pike D.G., Víkingsson G.A., Gunnlaugsson T., Øien N., 2009. A note on the distribution and abundance of blue whales (Balaenoptera musculus) in the Central and Northeast North Atlantic. NAMMCO Scientific Publications 7: 19. DOI: 10.7557/ 3.2703.

Pike D., Gunnlaugsson T., Mikkelsen B., Halldórsson S.D., Víkingsson G. 2019. Estimates of the abundance of cetaceans in the central North Atlantic based on the NASS Icelandic and Faroese shipboard surveys conducted in 2015. NAMMCO Scientific Publications, 11. DOI: 10.7557/3.4941

Polanowski A.M., Robbins J., Chandler D., Jarman S.N. 2014. Epigenetic estimation of age in humpback whales. Molecular Ecology Resources, 14: 976–987. DOI: 10.1111/1755-0998.12247

Polischuk S.C., Hobson K.A., Ramsay M.A. 2001. Use of stable-carbon and-nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Canadian Journal of Zoology, 79(3): 499-511. DOI: 10.1139/z01-007

Ramos R., González-Solís J. 2012. Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. Frontiers in Ecology and Environment, 10(5): 258–266. DOI:10.1890/110140

Ramp C., Hagen W., Palsbøll P., Bérubé M., Sears R. 2010. Age-related multi-year associations in female humpback whales (*Megaptera novaeangliae*). Behavioral Ecology & Sociobiology, 64: 1563–1576. DOI: 10.1007/s00265-010-0970-8.

Rees C.E., Jenkins W.J., Monster J. 1978. The sulphur isotopic composition. Geochimica et Cosmochimica Acta, 42(65): 377-381.

Reeves R., Notarbartolo di Sciara G. (compilers and editors). 2006. The status and distribution of cetaceans in the Black Sea and Mediterranean Sea. IUCN Centre for Mediterranean Cooperation, Malaga, Spain. 137 pp.

Richard G., Titova O.V., Fedutin I.D., Steel D., Meschersky I.G., Hautin M., Burdin A.M., Hoyt E., Filatova O.A., Jung J-L. 2018. Cultural transmission of fine-scale fidelity to feeding sites may shape Humpback whale genetic diversity in Russian Pacific waters. Journal of Heredity, 109(7): 724–734. DOI: 10.1093/jhered/esy033

Richards M.P., Fuller B.T., Sponheimer M., Robinson T., Ayliffe L. 2003. Sulphur isotopes in palaeodietary studies: a review and results from a controlled feeding experiment. International Journal of Osteoarchaeology, 13: 37 – 45. DOI: 10.1002/oa.654

Riofrío-Lazo M., Aurioles-Gamboa, D. 2013. Timing of isotopic integration in marine mammal skull: comparative study between calcified tissues. Rapid Communications in Mass Spectrometry, 27: 1076–1082. DOI: 10.1002/rcm.6556

Rita D. 2021. Estructura y migración del rorcual común del Atlántico nororiental establecido mediante trazadores químicos. Universitat de Barcelona, Tesis doctoral.

Robbins J., Dendanto D., Giard J., Panigada S., Sears R., Zanardelli M. 2007. Photo-ID studies of fin whales in the North Atlantic Ocean and the Mediterranean Sea. Report of the Scientific Committee of the International Whaling Commission SC/59/PF11, 1(4).

Romagosa M., Pérez-Jorge S., Cascão I., Mouriño H., Lehodey P., Pereira A., Marques T.A., Matias L., Silva M.A., 2021. Food talk: 40-Hz fin whale calls are associated with prey biomass. Proceedings of the Royal Society B, 288: 20211156. DOI: 10.1098/rspb.2021.1156

Rosel P.E., Wilcox L.A., Yamada T.K., Mullin K.D. 2021. A new species of baleen whale (*Balaenoptera*) from the Gulf of Mexico, with a review of its geographic distribution. Marine Mammal Science, 37: 577–610. DOI: 10.1111/mms.12776

Sanpera C., Aguilar A. 1992. Modern whaling off the Iberian Peninsula during the 20th century. Report of the International Whaling Commission, 42: 723–730

Sanpera C., González M., Jover L. 1996. Heavy metals in two populations of North Atlantic fin whales (*Balaenoptera physalus*). Environmental Pollution, 91(3): 299-307

Sasaki T., Nikaido M., Hamilton H., Goto M., Kato H., Kanda N., Pastene L.A., Cao Y., Fordyce R.E., Hasegawa M., Okada N. 2005. Mitochondrial Phylogenetics and Evolution of Mysticete Whales. Systematic Biology, 54(1): 77–90. DOI: 10.1080/10635150590905939

Saxanov S., Berg P., Brutlag D.L. 2006. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. Proceedings of the National Academy of Science of the United States of America, 103 (5): 1412-1417. DOI: 10.1073/pnas.0510310103

Sears R., Perrin W.F. 2018. Blue whale. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals (Third edition): 110–114

Sigurjónsson J., Víkingsson G.A. 1992. Investigations on the ecological role of cetaceans in Icelandic and adjacent waters. ICES CM 1992/N24: 23 pp.

Sigurjónsson J. 1995. On the life history and autecology of North Atlantic rorquals. Blix A.S., Walløe L., Ulltang Ø. (Eds.), Whales, Seals, Fish and Man, Elsevier Science, New York, USA: 425-441

Sigurjónsson J., Víkingsson, G.A. 1997. Seasonal abundance of and estimated food consumption by cetaceans in Icelandic and adjacent waters. Journal of Northwest Atlantic Fishery Science, 22: 271–287.

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(10): e76507. DOI: 10.1371/journal.pone.0076507

Silva M.A., Borrell A., Prieto R., Gauffier P., Bérubé M., Palsbøl P.J., Colaço A. 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. Royal Society open Science, 6: 181800. DOI: 10.1098/rsos.181800

Símonarson L.A., Eiríksson J., Knudsen K.L. 2020. The marine realm around Iceland – A review of biological research. In J. Eiríksson and L.A. Símonarson (Eds.), Pacific – Atlantic Mollusc migration. Cham, Switzerland: Springer. Topics in Geobiology, 52, Chapter 2.

Skaug H.J., Daníelsdóttir A., Víkingsson G.A., 2006. Relatedness of North Atlantic fin whales. IWC/SC/58/PFI9.

Skaug H.G., Berubé M., Palsboll P.J. 2010. Detecting dyads of related individuals in large collections of DNA-profiles by controlling the false discovery rate. Molecular Ecology Resources, 10: 693–700. DOI: 10.1111/j.1755-0998.2010.02833.x

Slater G.J., Price S.A., Santini F., Alfaro M.E. 2010. Diversity versus disparity and the radiation of modern cetaceans. Proceedings Royal Society B, 277: 3097–3104. DOI: 10.1098/rspb.2010.0408

Smith Z.D., Meissner A. 2013. DNA methylation: roles in mammalian development. Nature Reviews Genetics, 14: 204–220. DOI: 10.1038/nrg3354

Steeman M.E., Hebsgaard M.B., Fordyce R.E., Ho S.Y.W., Rabosky D.L., Nielsen R., Ragbek C., Glenner H., Sørensen M.V., Willerslev E. 2009. Radiation of Extant Cetaceans Driven by Restructuring of the Oceans. Systematic Biology, 58(6): 573–585. DOI: 10.1093/sysbio/syp060

Stefansdottir L., Solmundsson J., Marteinsdottir G., Kristinsson K., Jonasson, J.P. 2010. Groundfish species diversity and assemblage structure in Icelandic waters during recent years of warming. Fisheries Oceanography, 19 (1): 42–62. DOI: 10.1111/j.1365-2419.2009.00527.x

Stern S.J., Friedlaender A.S. 2018. Migration and movement. In Perrin W. F., Würsig B., Thewissen J.G.M. (Eds.), Encyclopedia of Marine Mammals (Third Edition): 602 – 606.

Strauss H. 2004. 4 Ga of seawater evolution: Evidence from the sulfur isotopic composition of sulfate. In Amend J.P., Edwards K.J., Lyons T.W. (eds). Sulfur biogeochemistry — Past and present. DOI: 10.1130/0-8137-2379-5.195

Tanabe A., Shimizu R., Osawa Y., Suzuki M., Ito S., Goto M., Pastene L.A., Yoshihiro F., Sahara H. 2020. Age estimation by DNA methylation in the Antarctic minke whale. Fisheries Science, 86(1): 35–41. DOI: 10.1007/s12562-019-01371-7

Tiedemann R., Tiedemann M.R., Gunnlaugsson Þ., Pampoulie C., Víkingsson G.A. 2012. Finding relatives among North Atlantic common minke whales (*Balaenoptera acutorostrata*) based on microsatellite data: the relationship between false discovery rate (FDR) and detection power. SC/65b/RMP05.

Tost J., Gut I.G. 2007. DNA methylation analysis by pyrosequencing. Nature Protocols, 2(9): 2265-2275. DOI: 10.1038/nprot.2007.314

Valdimarsson H., Astthorsson O.S., Palsson J. 2012. Hydrographic variability in Icelandic waters during recent decades and related changes in distribution of some fish species. ICES Journal of Marine Science, 69: 816–825. DOI: 10.1093/icesjms/fss027

Vander Zanden M.J., Clayton M.K., Moody E.K., Solomon C.T., Weidel B.C. 2015. Stable isotope turnover and half-life in animal tissues: A literature synthesis. PLoS ONE, 10(1): e0116182. DOI: 10.1371/journal.pone.0116182

Verhoeven K.J.F., Vonholdt B.M., Sork V.L. 2016. Epigenetics in ecology and evolution: what we know and what we need to know. Molecular Ecology, 25: 1631–1638. DOI: 10.1111/mec.13617

Vighi M., Borrell A., Aguilar A. 2016. Stable isotope analysis and fin whale subpopulation structure in the eastern North Atlantic. Marine Mammal Science, 32(2): 535–551. DOI: 10.1111/mms.12283

Víkingsson G.A. 1997. Feeding of fin whales (*Balaenoptera physalus*) off Iceland – diurnal and seasonal variation and possible rates. Journal of Northwest Atlantic Fishery Science, 22: 77–89. DOI: 10.2960/J.v22.a7

Víkingsson G.A., Gunnlaugsson Th. 2005. Stock structure of fin whales (*Balaenoptera physalus*) in the North Atlantic: Indications from non-genetic data. IWC/SC/57/PFI3. Available from the IWC. https://iwc.int/home/.

Víkingsson G.A., Pike D.G., Desportes G., Øien N., Gunnlaugsson T., Bloch D. 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 Scientific Publications, 7: 49-72. DOI: 10.7557/3.2705.

Víkingsson G.A., Elvarsson B.T., Ólafsdóttir D., Sigurjónsson J., Chosson V., Galan A. 2014. Recent changes in the diet composition of common minke whales (*Balaenoptera acutorostrata*) in Icelandic waters. A consequence of climate change? Marine Biology Research, 10(2): 138–152. DOI: 10.1080/17451000.2013.793812

Víkingsson G.A., Pike D.P., Valdimarsson H., et al. 2015. Distribution, abundance, and feeding ecology of baleen whales in Icelandic waters: have recent environmental changes had an effect? Frontiers in Ecology and Evolution, 3: 6. DOI: 10.3389/fevo.2015.00006

Visser F., Hartman K.L., Pierce G.J., Valavanis V.D., Huisman J. 2011. Timing of migratory baleen whales at the Azores in relation to the North Atlantic spring bloom. Marine Ecology Progress Series, 440:.267-279. DOI: 10.3354/meps09349

Wada S., Oishi M., Yamada T.K. 2003. A newly discovered species of living baleen whale. Nature, 426: 278 – 281. DOI: 10.1038/nature02103

Wang B., Yang W., McKittrick J., Meyers M.A. 2016. Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. Progress in Materials Science, 76: 229–318. DOI: 10.1016/j.pmatsci.2015.06.001

Watson H., Powell D., Salmón P., Jacobs A., Isaksson C. 2021. Urbanization is associated with modifications in DNA methylation in a small passerine bird. Evolutionary Applications, 14: 85–98. DOI: 10.1111/eva.13160

Webb E.C., Newton J., Lewis J., Stewart A., Miller B., Tarlton J.F., Evershed R.P. 2017. Sulphur-isotope compositions of pig tissues from a controlled feeding study. STAR:

Science & Technology of Archaeological Research, 3(1): 71 – 79. DOI: 10.1080/20548923.2017.1368821

Weinrich M.T. 1991. Stable social associations among humpback whales (Megaptera novaeangliae) in the southern Gulf of Maine. Canadian Journal of Zoology, 69: 3012-3018. DOI: 10.1139/z91-425.

Weyrich A., Lenz D., Jeschek M., Chung T.H., Rübensam K., Göritz F., Jewgenow K., Fickel J. 2016. Paternal intergenerational epigenetic response to heat exposure in male Wild guinea pigs. Molecular Ecology, 25: 1729–1740. DOI: 10.1111/mec.13494

Whitaker J.M., Welsh A.B., Hondorp D.W., Boase J.C., Merovich G.T., Welsh S., Krueger C. 2018. Variation in DNA methylation is associated with migratory phenotypes of lake sturgeon *Acipenser fulvescens* in the St. Clair River, MI, USA. Journal of Fish Biology, 93: 942–951. DOI: 10.1111/jfb.13804

Whitehead H., Rendell L. 2015. The Cultural Lives of Whales and Dolphins, Chicago: University of Chicago Press. DOI: 10.7208/9780226187426

Wilson V.L., Smith R.A., Mag S., Cutler R.G. 1987. Genomic 5-methyldeoxycytidine decreases with age. The Journal of Biological Chemistry, 262: 9948-9951.

Wright D.L., Witteveen B., Wynne K., Horstmann-Dehn L. 2016. Fine-scale spatial differences in humpback whale diet composition near Kodiak, Alaska. Marine Mammal Science, 32 (3): 1099–1114. DOI: 10.1111/mms.12311

Zhai L., Gudmundsson K., Miller P., Peng W., Gudfinnsson H., Debes H., Hátún H., White N.A., Hernández Walls R., Sathyendranath S., Platt T. 2012. Phytoplankton phenology and production around Iceland and Faroe. Continental Shelf Research, 37: 15–25. DOI: 10.1016/j.csr.2012.01.013.

von Ziegesar O., Gill S., Goodwin B. 2021. Long-term associations and insights on social structure of the humpback whales in Prince William Sound, Alaska. Long-term associations and social structure of Humpback whales. bioRxiv preprint. DOI: 10.1101/2020.03.02.972828