Evaluación interdisciplinar de la presencia Y distribución de las basuras marinas (macro-residuos flotantes, microplásticos y aditivos plásticos), y de sus impactos en vertebrados marinos

Odei Garcia Garin

EVALUACIÓN INTERDISCIPLINAR DE LA PRESENCIA Y DISTRIBUCIÓN DE LAS BASURAS MARINAS (MACRO-RESIDUOS FLOTANTES, MICROPLÁSTICOS Y ADITIVOS PLÁSTICOS), Y DE SUS IMPACTOS EN VERTEBRADOS MARINOS

Odei Garcia-Garin

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Facultat de Biologia
Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
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Evaluación interdisciplinar de la presencia y distribución de las basuras marinas (macro-residuos flotantes, microplásticos y aditivos plásticos), y de sus impactos en vertebrados marinos

Memoria presentada por
Odei Garcia Garin
Para optar al grado de doctor por la Universidad de Barcelona

Doctorando

Odei Garcia Garin

Directora y tutora

Dra. Asunción Borrell Thió

Directora

Dra. Morgana Vighi

We must remember: ... Everything is interlinked – the global commons and global well-being. That means we must act more broadly, more holistically, across many fronts, to secure the health of our planet on which all life depends…

UNEP (2021)

AGRADECIMIENTOS

Después de más de 4 años de tesis doctoral, las personas que me han ayudado, que se han interesado por el trabajo que estaba haciendo, que han compartido su valioso tiempo conmigo, son múltiples y diversas y, muy probablemente, será difícil constatar en unos pocos párrafos lo agradecido que estoy a todas ellas.

Primer de tot, volia agrair a la Núria per haver estat sempre allà, tan en els bons moments de la tesi, com en els no tan bons. Per escoltar-me sempre, aconsellar-me i donar-me ànims. Aquesta tesi no hauria sigut possible sense tu, ja que ha anat agafant forma al mateix temps que la nostra relació brillava una mica més cada dia, fins anar a tota vela.

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ÍNDICE

ABSTRACT

Marine litter is increasing exponentially in the seas and oceans of the world. In recent years, its threats to marine fauna and marine ecosystems in general have been reported and documented. For these reasons, mitigation measures are being adopted and monitoring programmes are being set in place to determine accumulation zones and evaluate the effectiveness of adopted measures. However, the information needed to determine baseline levels of marine litter and to improve and standardize monitoring methodologies is still poor. The general objective of this thesis is to investigate marine litter through an interdisciplinary approach to gain a broader perspective of its potential effects on marine vertebrates. In the first chapter, floating marine macro-litter is investigated using aerial photography from drones; floating marine macro-litter and marine megafauna are observed with a combined visual- and photographic-based approach from aircraft, and a deep learning model is developed to detect and quantify the floating marine macro-litter in aerial images. Results reveal that remote sensing techniques are as effective or more effective than visual techniques in detecting floating marine macro-litter and marine megafauna, and the deep learning model, installed in a web application, achieves an accuracy of 81%. In the second chapter, three potential bioindicator species (*i.e.*, the bogue (*Boops boops*), the fin whale (*Balaenoptera physalus*) and the Antarctic fur seal (*Arctocephalus gazella*)) are analysed to determine the occurrence of microplastics. Results reveal the occurrence of microplastics in 46% of the bogues sampled from the Catalan coast and in 52% of the North Atlantic fin whales, respectively, while no microplastics are found in Antarctic fur seal scats, suggesting that the waters of the Bransfield Strait have very low levels of plastic contamination. The third chapter aims to determine the concentration of plastic additives in samples of marine vertebrate tissues, analyse its relationship with the occurrence of ingested microplastics and investigate the processes of bioaccumulation and biomagnification of these pollutants. Results show that the levels of organophosphate esters in the muscle of the bogues from the Mediterranean Sea are considerable but not of concern and do not relate to the occurrence of microplastics in the fish gastrointestinal tracts. In addition, organophosphate esters and phthalates are detected in the muscle of North Atlantic fin whales, and although their levels do not seem of concern for the viability of the fin whale population, long-term exposure may lead to chronic toxicity. Finally, phthalate concentrations do not show intra-population or temporal differences in fin whales and organophosphate esters do not appear to bioaccumulate throughout the whale life-span and/or biomagnificate through the food web. The results of this thesis provide relevant information to improve marine litter monitoring programs as well as useful data to produce reference baseline values of floating marine macro-litter, microplastics and plastic additives.

RESUMEN

La basura marina está aumentando exponencialmente en los mares y océanos de todo el mundo. En los últimos años, se han reportado y documentado sus amenazas a la fauna marina y a los ecosistemas marinos en general. Por estas razones, se están fomentando medidas de mitigación y campañas de monitoreo para determinar cuáles son las zonas de acumulación de basura marina y evaluar la eficacia de las medidas propuestas. Sin embargo, todavía falta información para determinar los niveles basales de basura marina y mejorar las metodologías de monitoreo. El objetivo general de la presente tesis es estudiar la basura marina de manera interdisciplinar para tener una visión más amplia de sus potenciales efectos sobre los vertebrados marinos. En el primer capítulo, se investigan los macro-residuos flotantes mediante fotografía aérea desde drones y los macro-residuos flotantes y megafauna marina desde avionetas y se desarrolla un modelo de aprendizaje profundo para detectar y cuantificar los macro-residuos flotantes en las imágenes aéreas. Los resultados revelan que las técnicas de teledetección son igual o incluso más efectivas que las técnicas visuales para detectar macro-residuos flotantes y megafauna marina, y el modelo de aprendizaje profundo, instalado en una aplicación web, alcanza una efectividad del 81%. En el segundo capítulo, se utilizan tres especies potencialmente bioindicadoras (la boga (*Boops boops*), el rorcual común (*Balaenoptera physalus*) y el lobo marino antártico (*Arctocephalus gazella*)) para determinar la ocurrencia de microplásticos. Los resultados revelan la ocurrencia de microplásticos en el 46% y 52% de bogas de la costa catalana y rorcuales comunes del Atlántico Norte, respectivamente. Por lo contrario, no se encontraron microplásticos en los excrementos de lobo marino antártico, resultado que sugiere que las aguas del Estrecho de Bransfield presentan niveles muy bajos de contaminación plástica. En el tercer capítulo, se determina la concentración de aditivos plásticos en muestras de tejido de vertebrados marinos, para analizar su relación con las concentraciones de microplásticos e investigar los procesos de bioacumulación y biomagnificación de estos contaminantes. Los resultados muestran que las bogas del Mediterráneo presentan niveles considerables, pero no preocupantes, de ésteres organofosforados en sus tejidos musculares y que estas concentraciones no están relacionadas con la ocurrencia de microplásticos en sus tractos gastrointestinales. Además, los resultados muestran que los rorcuales comunes del Atlántico Norte presentan ésteres organofosforados y ftalatos en sus tejidos musculares y, aunque parece que los niveles no sean preocupantes para la viabilidad de sus poblaciones, una exposición a largo término podría desencadenar una toxicidad crónica. Además, parece ser que los ésteres organofosforados no se bioacumulan ni biomagnifican, y que los ftalatos no presentan diferencias intra-poblacionales ni temporales en rorcual común. En conjunto, los resultados de esta tesis proporcionan información de utilidad para mejorar los programas de monitoreo de basuras marinas y datos que pueden ser usados como referencia de los valores basales de macro-residuos flotantes, microplásticos y aditivos plásticos.

INFORME DE LAS DIRECTORAS DE TESIS

Como directoras de la tesis doctoral titulada “Evaluación interdisciplinar de la presencia y distribución de las basuras marinas (macro-residuos flotantes, microplásticos y aditivos plásticos), y de sus impactos en vertebrados marinos” realizada por Odei Garcia-Garin, detallamos en el siguiente informe la contribución del doctorando en cada uno de los nueve artículos que componen su tesis:

Capítulo 1.1: Garcia-Garin, O., Borrell, A., Aguilar, A., Cardona, L., & Vighi, M. (2020). Floating marine macro-litter in the North Western Mediterranean Sea: Results from a combined monitoring approach. *Marine Pollution Bulletin*, *159*, 111467. https://doi.org/10.1016/j.marpolbul.2020.111467

Contribución del doctorando: Participación en el diseño experimental y en el trabajo de campo, revisión de las imágenes, análisis estadísticos, y redacción del manuscrito.

Acerca de la revista: *Marine Pollution Bulletin*. En el Journal Citation Reports (JRC) de 2020 tiene un índice de impacto de 5,55. Se encuentra en el número 3 de 110 en el área de Biología marina y de agua dulce (1er cuartil).

Capítulo 1.2: Garcia-Garin, O., Aguilar, A., Borrell, A., Gozalbes, P., Lobo, A., Penadés-Suay, J., Raga, J. A., Revuelta, O., Serrano, M., & Vighi, M. (2020). Who’s better at spotting? A comparison between aerial photography and observer-based methods to monitor floating marine litter and marine mega-fauna. *Environmental Pollution*, *258, 113680*. https://doi.org/10.1016/j.envpol.2019.113680

Contribución del doctorando: Participación en el diseño experimental y en el trabajo de campo, revisión de las imágenes, análisis estadísticos, y redacción del manuscrito.

Acerca de la revista: *Environmental Pollution*. En el Journal Citation Reports (JRC) de 2020 tiene un índice de impacto de 8,07. Se encuentra en el número 23 de 274 en el área de Ciencias ambientales (1er cuartil).

Capítulo 1.3: Garcia-Garin, O., Monleón-Getino, T., López-Brosa, P., Borrell, A., Aguilar, A., Borja-Robalino, R., Cardona, L., & Vighi, M. (2021). Automatic detection and quantification of floating marine macro-litter in aerial images: Introducing a novel deep learning approach connected to a web application in R. *Environmental Pollution*, *273*, 116490. https://doi.org/10.1016/j.envpol.2021.116490

Contribución del doctorando: Participación en el diseño experimental y en el trabajo de campo, revisión de las imágenes, análisis estadísticos, y redacción del manuscrito.

Acerca de la revista: *Environmental Pollution*. En el Journal Citation Reports (JRC) de 2020 tiene un índice de impacto de 8,07. Se encuentra en el número 23 de 274 en el área de Ciencias ambientales (1er cuartil).

Capítulo 2.1: Garcia-Garin, O., Vighi, M., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., & Borrell, A. (2019). *Boops boops* as a bioindicator of microplastic pollution along the Spanish Catalan coast. *Marine Pollution Bulletin*, *149*, 110648. https://doi.org/10.1016/j.marpolbul.2019.110648

Contribución del doctorando: Participación en el diseño experimental y en la recolección de muestras, análisis de laboratorio y estadísticos, y redacción del manuscrito.

Acerca de la revista: *Marine Pollution Bulletin*. En el Journal Citation Reports (JRC) de 2019 tiene un índice de impacto de 4,05. Se encuentra en el número 4 de 107 en el área de Biología marina y de agua dulce (1er cuartil).

Capítulo 2.2: Garcia-Garin, O., Aguilar, A., Vighi, M., Víkingsson, G. A., Chosson, V., & Borrell, A. (2021). Ingestion of synthetic particles by fin whales feeding off Western Iceland in summer. *Chemosphere*, *279*, 130564. https://doi.org/10.1016/j.chemosphere.2021.130564

Contribución del doctorando: Participación en el diseño experimental, en los análisis de laboratorio y estadísticos, y en la redacción del manuscrito.

Acerca de la revista: *Chemosphere*. En el Journal Citation Reports (JRC) de 2020 tiene un índice de impacto de 7,09. Se encuentra en el número 30 de 274 en el área de Ciencias ambientales (1er cuartil).

Capítulo 2.3: Garcia-Garin, O., García-cuevas, I., Drago, M., Rita, D., Parga, M., Gazo, M., & Cardona, L. (2020). No evidence of microplastics in Antarctic fur seal scats from a hotspot of human activity in Western Antarctica. *Science of the Total Environment*, *737*, 140210. https://doi.org/10.1016/j.scitotenv.2020.140210

Contribución del doctorando: Participación en el diseño experimental, análisis de laboratorio, y redacción del manuscrito.

Acerca de la revista: *Science of the Total Environment*. En el Journal Citation Reports (JRC) de 2020 tiene un índice de impacto de 7,96. Se encuentra en el número 25 de 274 en el área de Ciencias ambientales (1er cuartil).

Capítulo 3.1: Garcia-Garin, O., Vighi, M., Sala, B., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., Eljarrat, E., & Borrell, A. (2020). Assessment of organophosphate flame retardants in Mediterranean *Boops boops* and their relationship to anthropization levels and microplastic ingestion. *Chemosphere*, *252*, 126569. https://doi.org/10.1016/j.chemosphere.2020.126569

Contribución del doctorando: Participación en el diseño experimental y en la recolección de muestras, análisis de laboratorio y estadísticos, y redacción del manuscrito.

Acerca de la revista: *Chemosphere*. En el Journal Citation Reports (JRC) de 2020 tiene un índice de impacto de 7,09. Se encuentra en el número 30 de 274 en el área de Ciencias ambientales (1er cuartil).

Capítulo 3.2: Garcia-Garin, O., Sala, B., Aguilar, A., Vighi, M., Víkingsson, G. A., Chosson, V., Eljarrat, E., & Borrell, A. (2020). Organophosphate contaminants in North Atlantic fin whales. *Science of the Total Environment*, *721*, 137768. https://doi.org/10.1016/j.scitotenv.2020.137768

Contribución del doctorando: Participación en el diseño experimental, análisis de laboratorio y estadísticos, y redacción del manuscrito. La coautora Berta Sala ha utilizado este trabajo para la elaboración de su tesis doctoral.

Acerca de la revista: *Science of the Total Environment*. En el Journal Citation Reports (JRC) de 2020 tiene un índice de impacto de 7,96. Se encuentra en el número 25 de 274 en el área de Ciencias ambientales (1er cuartil).

Capítulo 3.3: Garcia-Garin, O., Sahyoun, W., Net, S., Vighi, M., Aguilar, A., Ouddane, B., Víkingsson, G. A., Chosson, V., & Borrell, A. (2022). Intrapopulation and temporal differences of phthalate concentrations in North Atlantic fin whales (*Balaenoptera physalus*). *Chemosphere*, *300*, 134453. https://doi.org/10.1016/j.chemosphere.2022.134453

Contribución del doctorando: Participación en el diseño experimental, análisis de laboratorio y estadísticos, y redacción del manuscrito.

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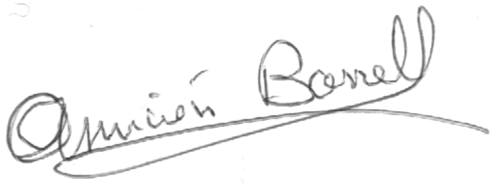
Barcelona, 25 de marzo de 2022

Firma de las directoras:



Dra. Asunción Borrell Thió
Departamento de Biología
Evolutiva, Ecología y Ciencias
Ambientales Facultad de Biología
Universidad de Barcelona

Dra. Morgana Vighi
Tethys Research Institute, Acquario
Civico - viale G.B. Gadio 2, 20121
Milano, Italy

INTRODUCCIÓN GENERAL

1. Distribución, composición y abundancia de la basura marina

La basura marina se puede definir como cualquier material sólido y persistente, que ha sido manufacturado o procesado, y que se ha abandonado, tirado o perdido en el medio marino o costero (UNEP, 2005). La presencia de basura marina se ha constatado en todos los océanos del mundo (Cózar et al., 2014), desde el Ártico (Kanhai et al., 2018; Kühn et al., 2018), hasta la Antártida (Suaria et al., 2020; Waller et al., 2017). No obstante, las densidades máximas se han registrado cerca de los principales giros oceánicos (p. ej., el *Great Pacific Garbage Patch*; Lebreton et al., 2018) o en mares semi-cerrados como el Mediterráneo (Cózar et al., 2015; Ruiz-Orejón et al., 2016, 2018, 2019; Suaria et al., 2014) (Figura 1). La basura marina se distribuye en todos los compartimentos de los mares y océanos, desde la línea de costa (Gonçalves et al., 2020a; Nelms et al., 2017), los sedimentos (Alomar et al., 2016; Munari et al., 2017) y bentos (Dominguez-Carrió et al., 2020), la columna de agua (Choy et al., 2019), y hasta la superficie (Arcangeli et al., 2018, 2020; de Haan et al., 2019; Lambert et al., 2020).

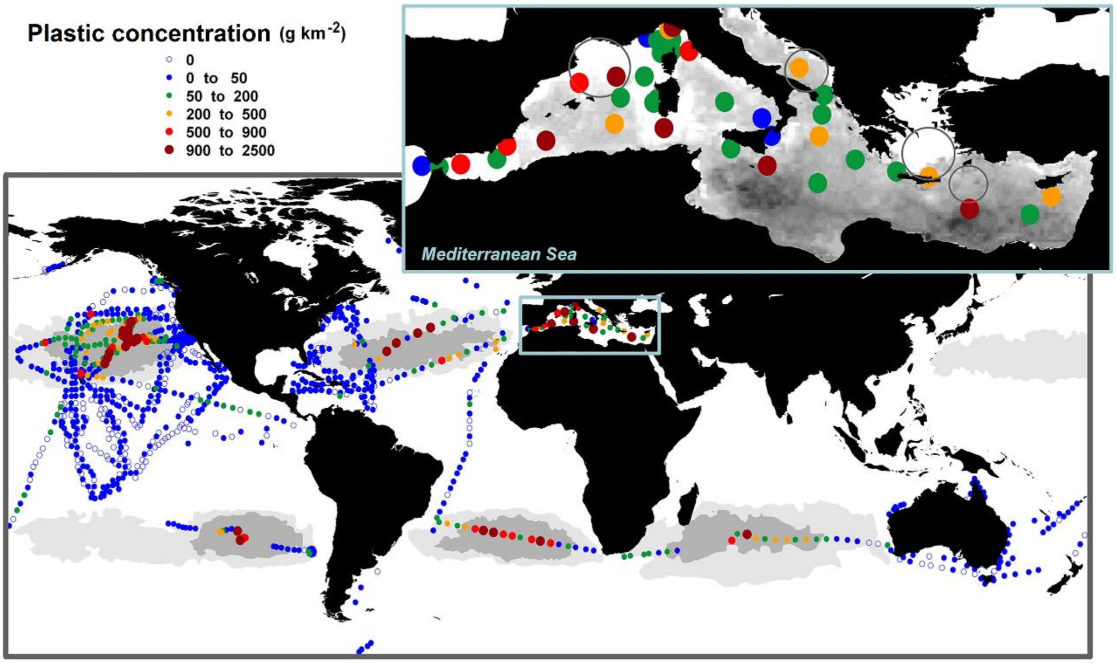


Figura 1: Concentración de basura plástica flotante en la superficie del Mar Mediterráneo y de los océanos. Figura adaptada de Cózar et al. (2015).

La basura marina, que puede estar compuesta por plástico en más del 80% (Arcangeli et al., 2018; Galgani et al., 2015; UNEP/ MAP, 2015), se categoriza en macro- (>2,5 cm), meso- (2,5 cm-5 mm), micro- (5 mm-1 µm) y nano- (<1 µm) (Arthur et al., 2009; Dawson et al., 2018; Galgani et al., 2013; GESAMP, 2019). La mayoría de microplásticos y nanoplásticos provienen de la fragmentación de los macroplásticos (p. ej., botellas, redes, bolsas; UNEP, 2016). La deterioración y fragmentación se produce principalmente por los efectos de la luz ultravioleta, las olas y los enzimas de los organismos (Figura 2). Además, los plásticos llevan incorporados aditivos (p. ej., ftalatos, bisfenoles, polibromodifenil éteres (PBDEs), ésteres organofosforados) para mejorar algunas de sus propriedades como dureza, flexibilidad, resistencia, etc. (Avio et al., 2017). También, la basura marina puede actuar como vector de transporte de otros contaminantes, como metales pesados, bifenilos policlorados (PCBs), pesticidas (p. ej., DDTs) e hidrocarburos aromáticos policíclicos (Avio et al., 2017).

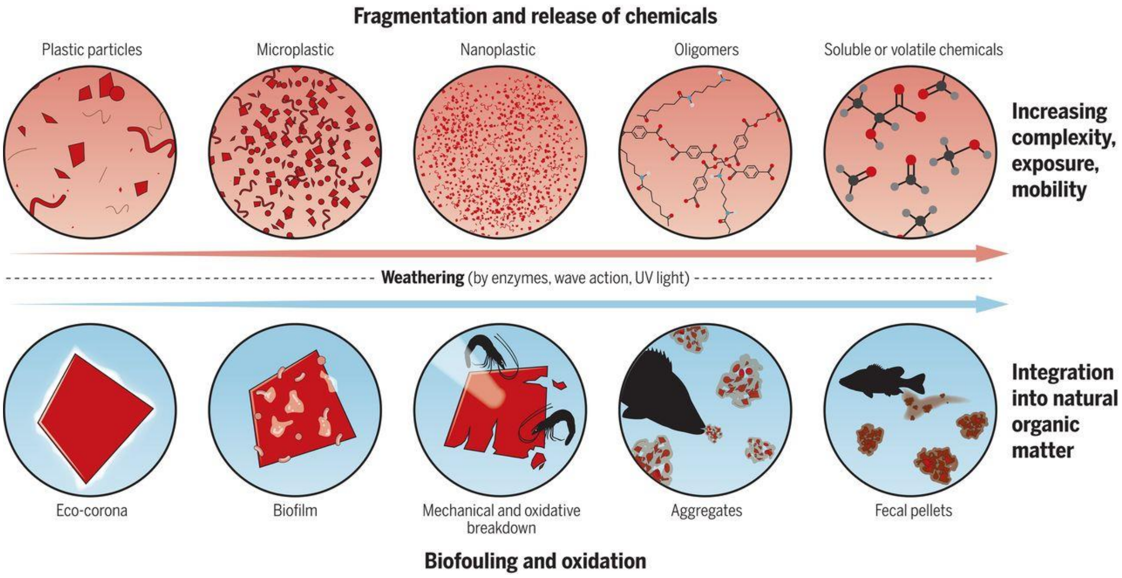


Figura 2: Procesos de deterioración y fragmentación de los plásticos en el mar. Figura adaptada de MacLeod et al. (2021).

En 2015 se estimó la presencia de 5,25 trillones de partículas de basura flotante en los mares y océanos de todo el mundo (Eriksen et al., 2014), a los que anualmente se sumarían entre 4,8 y 12,7 millones de toneladas de plástico (Jambeck et al., 2015). En el mar Mediterráneo, uno de los mares más contaminados del mundo, se han detectado densidades de basura de hasta 600 objetos km^{-2} (UNEP/MAP, 2015), y en 2020 se estimó la presencia de 11,5 millones de macro-residuos marinos flotando en su superficie (Lambert et al., 2020). Las vías de entrada son diversas, desde la basura que se tira al suelo o cae involuntariamente y es transportada por los vientos hasta el mar, la que es transportada por las aguas residuales, hasta la que llega al mar debido a su mala gestión (Duckett et al., 2015; Galgani et al., 2013), además de toda la basura proveniente del sector pesquero y del tráfico naval (Galgani et al., 2013). Como consecuencia de la alta resistencia y perdurabilidad de los plásticos, la cantidad de residuos plásticos que se están acumulando en los ecosistemas marinos está incrementando exponencialmente (Barnes et al., 2009).

Todo esto hace que la basura marina represente hoy en día una de las principales amenazas para la fauna que habita los mares y océanos de la Tierra.

2. Impacto de la basura sobre la fauna marina

El impacto de la basura sobre la fauna marina se ha observado principalmente por cuatro vías.

La primera vía es la ingesta de basura marina de manera directa, o indirecta a través de una presa contaminada (Deudero et al., 2015; Figura 3). La ingesta de basura se ha observado en más de 331 especies marinas (Kühn et al., 2015), incluyendo poliquetos (Wright et al., 2013), moluscos (Digka et al., 2018), crustáceos (Desforges et al., 2015), peces (Fossi et al., 2014; Tsangaris et al., 2020), tortugas (Domènech et al., 2019), aves (Codina-García et al., 2013) y mamíferos (Besseling et al., 2015; Puig-Lozano et al., 2018; De Stephanis et al., 2013; Zantis et al., 2021a). Esta vía puede provocar un debilitamiento del animal, limitando su capacidad de alimentación debido a la obstrucción y daño de su sistema digestivo, y puede llegar a causarle la muerte (Gall y Thompson, 2015).

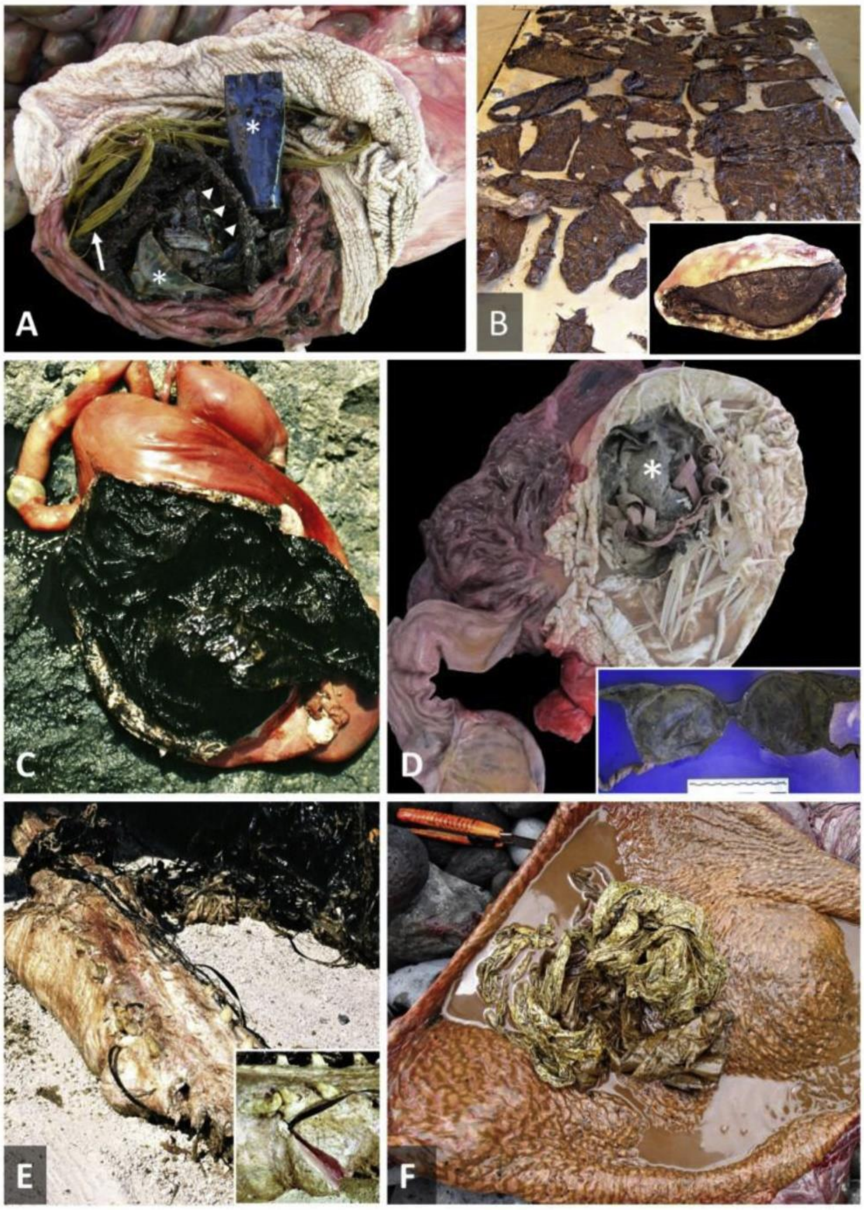


Figura 3: Obstrucción del estómago por basuras marinas en diferentes especies de cetáceos de las Islas Canarias. Figura adaptada de Puig-Lozano et al. (2018). A: *Grampus griseus*; B: *Stenella frontalis*; C: *Stenella frontalis*; D: *Tursiops truncatus*; E: *Physeter macrocephalus*; F: *Ziphius cavirostris.*

La segunda vía es el enredo de la fauna marina con la basura (p. ej., en redes, anillas de plástico, hilos, bolsas) (Gall y Thompson, 2015; Stelfox et al., 2016; Figura 4).

Esta vía se ha observado en más de 334 especies marinas (Kühn et al., 2015). El enredo con la basura marina puede limitar los movimientos del animal, causarle heridas y, también, puede llegar a causarle la muerte (Gall y Thompson, 2015). La fauna marina más propicia a enredarse con basura es habitualmente la que presenta un tamaño más grande, como los peces, tortugas, aves y mamíferos (Deudero et al., 2015). No obstante, se ha observado que algunos crustáceos y otras especies más pequeñas también son potenciales víctimas de enredarse con la basura marina (Kühn et al., 2015).

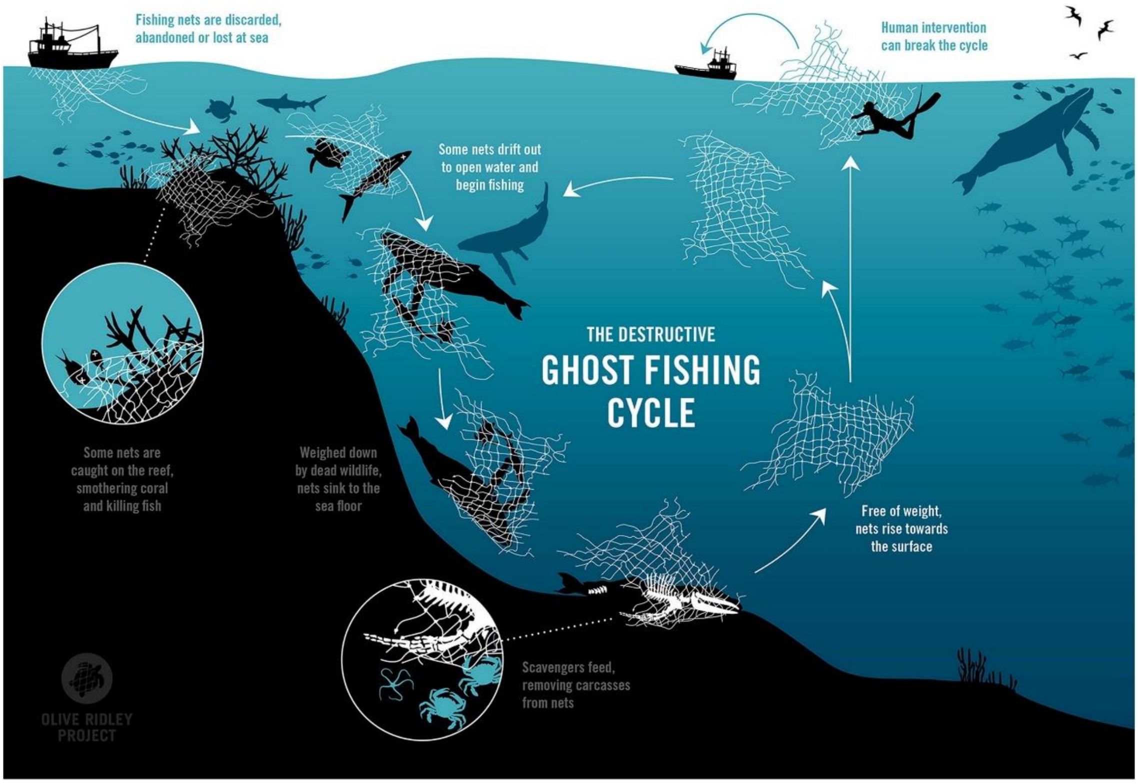


Figura 4: Enredo de la fauna marina con redes de pesca abandonadas. Figura adaptada de Stelfox et al. (2016).

Los aditivos y contaminantes acoplados a la basura conforman la tercera vía. Estos compuestos pueden acumularse en los órganos de la fauna marina y alterar sus procesos biológicos, actuando como disruptores endocrinos o como depresores del sistema inmunitario (Aguilar y Borrell, 1994; Mathieu-Denoncour, 2015; Talsness et al., 2009). Los aditivos plásticos se han detectado en varias especies de fauna marina, incluyendo peces (Kim et al., 2011), aves (Hardesty et al., 2015), tortugas (Savoca et al., 2018) y mamíferos (Bartalini et al., 2019; Hallanger et al., 2015; Sala et al., 2019).

La cuarta vía de impacto está relacionada con la transformación y degradación de los hábitats donde viven las especies marinas (Carson et al., 2011; Richards y Beger, 2011). Se ha observado que los plásticos reducen la tasa de crecimiento de las algas debido a sus efectos tóxicos, y físicos, bloqueando la captación de luz. También, limitan la realización de la fotosíntesis, disminuyendo la cantidad de clorofila en las células de estos organismos (Kühn et al., 2015; Zhang et al., 2017). Además, los microplásticos pueden desequilibrar las relaciones simbióticas entre antozoos y algas, penetrando en las células del antozoo y dañándolas (Okubo et al., 2018). Los corales obtienen energía gracias a esta relación simbiótica con las algas, y el debilitamiento de sus arrecifes podría causar una gran pérdida de biodiversidad (Richards y Beger, 2011). Por otra parte, la colonización de los plásticos por parte de la fauna marina, proceso llamado bioincrustación, puede conllevar que especies no indígenas invadan nuevos territorios o que plásticos de densidad baja como el polietileno o el polipropileno acaben sumergiéndose en el bentos marino (Kiessling et al., 2015; Subías-Baratau et al., 2022).

Todo esto hace que sea esencial llevar a cabo un monitoreo constante y global de la basura marina, para poder determinar su distribución, composición y abundancia y, de esta manera, poder implementar medidas efectivas para la mitigación de sus impactos (Galgani, 2019; GESAMP, 2015; UNEP, 2016).

3. Metodologías para monitorear la basura y su impacto en la fauna marina

Las metodologías usadas para el monitoreo de la basura marina son múltiples y específicas según el tipo de basura o compartimento marino que se quiere estudiar. Las más usadas son el muestreo mediante redes, el análisis de sedimentos, las observaciones visuales, el análisis de especies bioindicadoras y las técnicas de teledetección, además de los diferentes tipos de cromatografías para detectar los microplásticos y aditivos plásticos.

Las redes, por las cuales se filtra el agua marina, se han utilizado extensamente para determinar la densidad y composición de los macro- y micro-residuos marinos, tanto en la columna de agua, como en superficie (Camins et al., 2020; Cózar et al., 2014; de Haan et al., 2019; Eriksen et al., 2014; Lebreton et al., 2018; Suaria et al., 2016, 2020). El análisis de sedimentos también se ha utilizado para determinar la ocurrencia de microplásticos en playas y bentos marino (Bissen et al., 2020; Reed et al., 2018).

Tradicionalmente, también se han utilizado diversos tipos de plataformas de observación para monitorear los macro-residuos marinos flotantes. Las más utilizadas han sido las pequeñas embarcaciones (Di-Méglio & Campana, 2017), los ferris de pasajeros (Arcangeli et al., 2018, 2020) y otros tipos de embarcaciones (Suaria et al., 2020) o avionetas (Garaba et al., 2018; Lebreton et al., 2018). Los transectos visuales en playas o durante sus limpieza también se han utilizado para determinar la abundancia y distribución de la basura marina (Nelms et al., 2020; Willoughby et al., 1997).

Actualmente, las técnicas de teledetección están abriendo una nueva vía para el monitoreo de la basura marina. Tanto los sensores pasivos (p. ej., RGB vídeo, cámaras digitales, multiespectrales, híper-espectrales) como los sensores activos (p. ej., lídar, radar) pueden ser útiles para detectar la basura marina desde robots subacuáticos (Dominguez-Carrió et al., 2020), embarcaciones, y plataformas aéreas (p. ej., drones, avionetas, satélites) (Gonçalves et al., 2020a; Kikaki et al., 2020; Maximenko et al., 2019; Topouzelis et al., 2019; Veenstra et al., 2012). Para que los métodos de teledetección sean más efectivos, se han desarrollado diversos algoritmos para detectar automáticamente la marina en las imágenes, y se ha demostrado que los algoritmos de aprendizaje profundo basados en redes neuronales convolucionales ofrecen buenos resultados de detección (Kylili et al., 2019; Gonçalves et al., 2020a). A pesar de todos los avances significativos en la detección automática de la basura marina, los algoritmos actuales deben aún perfeccionarse para mejorar su efectividad (Martínez-Vicente et al., 2019; Maximenko et al., 2019).

También se han utilizado especies bioindicadoras para evaluar la contaminación por basura marina, tanto por macro-residuos (Digka et al., 2020; Domènech et al., 2019; Matiddi et al., 2017), como por micro-residuos (Bray et al., 2019), aditivos plásticos (Fossi et al., 2016) y otros contaminantes (Fossi y Depledge, 2014). Además, se han utilizado biomarcadores para determinar el impacto de la basura marina a nivel molecular, celular y de tejidos (Fossi et al., 2018). Los principales grupos de animales que se han utilizado como bioindicadores de contaminación por basura marina han sido los poliquetos, crustáceos, peces, tortugas, aves y mamíferos marinos (Fossi et al., 2018).

Finalmente, la metodología más usada para la detección de aditivos plásticos es el análisis en laboratorio por cromatografía de líquidos o gases acoplada con detección por espectrometría de masas u otros detectores selectivos. Para estos tipos de análisis, las muestras se tienen que extraer y purificar previamente (Bartalini et al., 2019; Fossi et al., 2014; Hao et al., 2018; Pang et al., 2016; Rodriguez et al., 2006; Sala et al., 2019; Wolschke et al., 2015). Por otro lado, la pirólisis-cromatografía de gases-espectrometría de masas es una técnica que ha sido propuesta recientemente para identificar y cuantificar microplásticos en muestras ambientales (Fischer y Scholz-Böttcher, 2017). En esta técnica los polímeros son convertidos a productos de menor peso molecular por la acción del calor. La composición y la abundancia relativa de los productos de la pirólisis son característicos para un polímero dado y su determinación permite la identificación de materiales que no pueden ser determinados de otra manera.

Todas estas técnicas de monitoreo se aplican con diferentes variaciones según el grupo de investigación, el proyecto de investigación, etc., es decir, no están completamente estandardizadas. Por esta razón, a menudo es complicado poder comparar los datos obtenidos entre diferentes estudios. El monitoreo de la densidad y de los patrones de distribución de la basura marina mediante metodologías estandardizadas (Van Sebille et al., 2020) es crucial para entender el alcance de esta amenaza (GESAMP, 2015; UNEP, 2016).

4. Especies modelos escogidas para la tesis

Para realizar las investigaciones de la presente tesis se han seleccionado 3 especies modelos en función de su relevancia en base a varios factores (distribución, facilidad de obtención y precio de las muestras, probabilidad de ingesta de basura marina, etc.).

La boga

La boga (*Boops boops*; Figura 5) es un pez bentopelágico que se distribuye desde Noruega hasta Angola, incluyendo el mar Negro y el mar Mediterráneo, donde su distribución es ubicua (FAO, 2020). Es una especie comestible y, por lo tanto, de fácil muestreo. Varios estudios avalan a la boga como especie bioindicadora de microplásticos en el medio marino del mar Mediterráneo, en primer lugar debido a que la ocurrencia de microplásticos en su tracto gastrointestinal es alta, probablemente a causa de sus hábitos alimenticios de succión de las presas, y en segundo lugar porque presenta una tracto gastrointestinal relativamente largo (unos 17 cm) (Bray et al., 2019; Tsangaris et al., 2020).

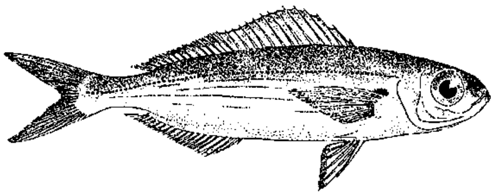


Figura 5: Boga (*Boops boops*).

El rorcual común del Atlántico norte

El rorcual común (*Balaenoptera physalus*; Figura 6) del Atlántico norte presenta un régimen migratorio anual que alterna zonas de latitud alta en verano (donde el alimento es abundante) con zonas de latitud baja en invierno (donde el alimento es escaso pero las temperaturas son favorables para la reproducción) (Aguilar y García-Vernet, 2018; Mizroch et al., 1984; Edwards et al., 2015). Las aguas de Islandia son una de las principales áreas de alimentación para el rorcual común, donde ingiere por filtración, principalmente, la especie de krill *Meganyctiphanes norvegica* (Víkingsson, 1997). Debido a estas características y al hecho que es una especie de vida longeva, es probable que el rorcual común esté expuesto a la ingesta de basura marina y, por lo tanto, puede ser un buen bioindicador de la ocurrencia de microplásticos y aditivos plásticos en el océano (Fossi et al., 2014, 2018). En el pasado, el rorcual común ya había sido usado como bioindicador de las características físico-químicas de las masas de agua oceánicas (Borrell et al., 2018).

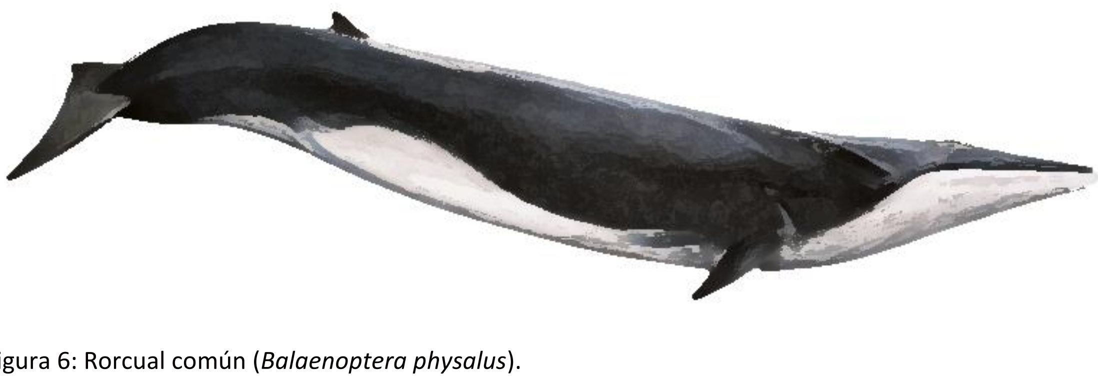


Figura 6: Rorcual común (*Balaenoptera physalus*).

El lobo marino de dos pelos antártico

El lobo marino de dos pelos antártico (en lo sucesivo, lobo marino antártico; *Arctocephalus gazella*; Figura 7) vive exclusivamente en el océano Austral. Esta especie fue muy explotada por la industria peletera durante la primera mitad del siglo XIX. Tras recuperarse notablemente, sus poblaciones están en regresión desde 2003. Se alimenta principalmente de krill antártico (*Euphausia superba*) y de peces mictófidos (Bonner, 1968; Davis et al., 2006; Goldsworthy et al., 1997), presas que son propensas a la ingestión de microplásticos (Desforges et al., 2015; Bernal et al., 2019). Los lobos marinos como grupo taxonómico han sido propuestos como bioindicadores de microplásticos (Pérez-Venegas et al., 2020), y se han encontrado microplásticos en heces de lobos marinos antárticos (Eriksson y Burton, 2003) a latitudes superiores al Frente Polar Antártico. Por todos estos factores, el lobo marino antártico puede ser un buen indicador de la ocurrencia de microplásticos en aguas antárticas.

Figura 7: Lobo marino de dos pelos antártico (*Arctocephalus gazella*).

5. Áreas de estudio

Para realizar las investigaciones de la presente tesis se han seleccionado 3 áreas de estudio (Figura 8) en función de su relevancia en base a varios factores (área poco estudiada, facilidad para obtener muestras, nivel de impacto antropogénico).

Las aguas de Islandia

Es un área poco antropizada donde se alimentan una gran diversidad de misticetos, como por ejemplo el rorcual común (García-Vernet et al., 2021). Esto hace que la obtención de muestras de rorcual común sea relativamente fácil y sea posible testar hipótesis relacionadas con la ingestión o absorción de microplásticos y aditivos plásticos mediante la filtración de agua o indirectamente mediante su presa principal, el krill *Meganyctiphanes norvegica.*

La costa española del Mediterráneo

Es un área muy urbanizada e industrializada, con grandes ciudades como Valencia y Barcelona (Liubartseva et al., 2018), aunque al mismo tiempo también presenta áreas marinas protegidas menos antropizadas (como el Cap de Creus o el

Delta de l'Ebre). Esta diversidad de presiones antrópicas produce que sea un área idónea para investigar y testar diversas hipótesis relacionadas con los residuos plásticos.

Islas Shetland del sur (Antártida)

Es una de las zonas más antropizadas de la Antártida (Hughes y Ashton, 2017; Waller et al., 2017), pero hay pocos estudios que hayan investigado los microplásticos presentes en la columna de agua de una de las zonas más prístinas del mundo. La colonia de lobos marinos antárticos de la isla Decepción (Hofmeyr, 2016) se alimenta en las aguas de las islas Shetland del sur durante el verano austral. Por este motivo, el análisis de sus heces para detectar microplásticos puede informar de los microplásticos presentes en aguas antárticas.

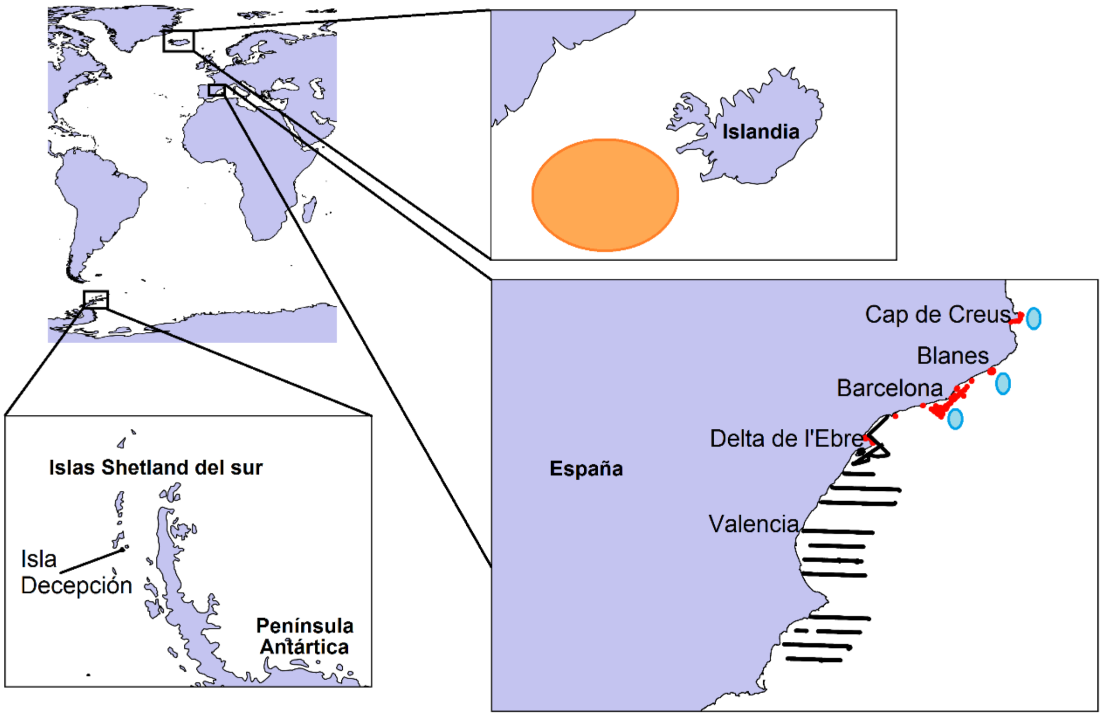


Figura 8: Áreas donde se han llevado a cabo las investigaciones de la presente tesis. En naranja y en azul están representadas las áreas dónde se recogieron las muestras de rorcual común y boga, respectivamente. En rojo y negro están representados los transectos hechos en dron y avioneta, respectivamente. Los excrementos de lobo marino antártico se recogieron en la isla Decepción (Islas Shetland del Sur).

OBJETIVOS

Esta tesis se centra en el estudio de métodos para investigar la presencia y distribución de la basura marina y su impacto en los vertebrados marinos. Se ha observado que la basura marina presenta una distribución, una composición y una abundancia muy heterogéneas y que puede interactuar y afectar los vertebrados marinos por diferentes vías. Por estas razones, el **objetivo general** de esta tesis se ha establecido en estudiar diferentes aspectos relacionados con las basuras marinas para poder tener una visión amplia de la problemática y, de esta manera, poder extraer unas conclusiones contextualizadas de sus potenciales impactos sobre los vertebrados marinos. Los resultados obtenidos en esta tesis podrán servir para mejorar las técnicas de monitoreo de la basura marina, incrementar el conocimiento actual de su distribución, composición y abundancia y para entender mejor su impacto en los vertebrados marinos.

Para conseguir este objetivo general se han planteado varios **objetivos específicos**:

- Testar la eficacia de drones y avionetas para el monitoreo de la basura flotante y de los vertebrados marinos, y desarrollar algoritmos para su detección automática en las imágenes aéreas.
- Obtener información sobre la contaminación por microplásticos mediante el análisis de especies potencialmente bioindicadoras.
- Determinar la concentración de aditivos plásticos en muestras de tejido de vertebrados marinos, su relación con las concentraciones de microplásticos ingeridos y los potenciales procesos de bioacumulación y biomagnificación de estos contaminantes.

Los objetivos de esta tesis fueron logrados mediante el estudio de los macro-residuos flotantes, microplásticos y aditivos plásticos utilizando drones, avionetas, análisis de especies bioindicadoras de vertebrados marinos, y análisis químicos, a lo largo de la costa mediterránea de España, las costas de Islandia y Antártida como casos de estudio (Figura 9).

La tesis se divide en 3 capítulos principales, según el aspecto de la basura marina estudiado, y cada capítulo se divide en subcapítulos según el caso de estudio:

1. **Macro-residuos flotantes:** utilizar tecnologías de teledetección para investigar la abundancia y distribución de basura flotante y de vertebrados marinos.
   1.1. **Drones:** testar el uso de drones para el monitoreo de la basura marina flotante (Capítulo 1.1).
   1.2. **Avionetas:** testar el uso de avionetas para el monitoreo de la basura marina flotante y de la fauna marina (Capítulo 1.2).
   1.3. **Algoritmos de detección automática**: desarrollar algoritmos para detectar automáticamente la basura marina flotante en las imágenes aéreas tomadas con los vehículos antes mencionados (Capítulo 1.3).

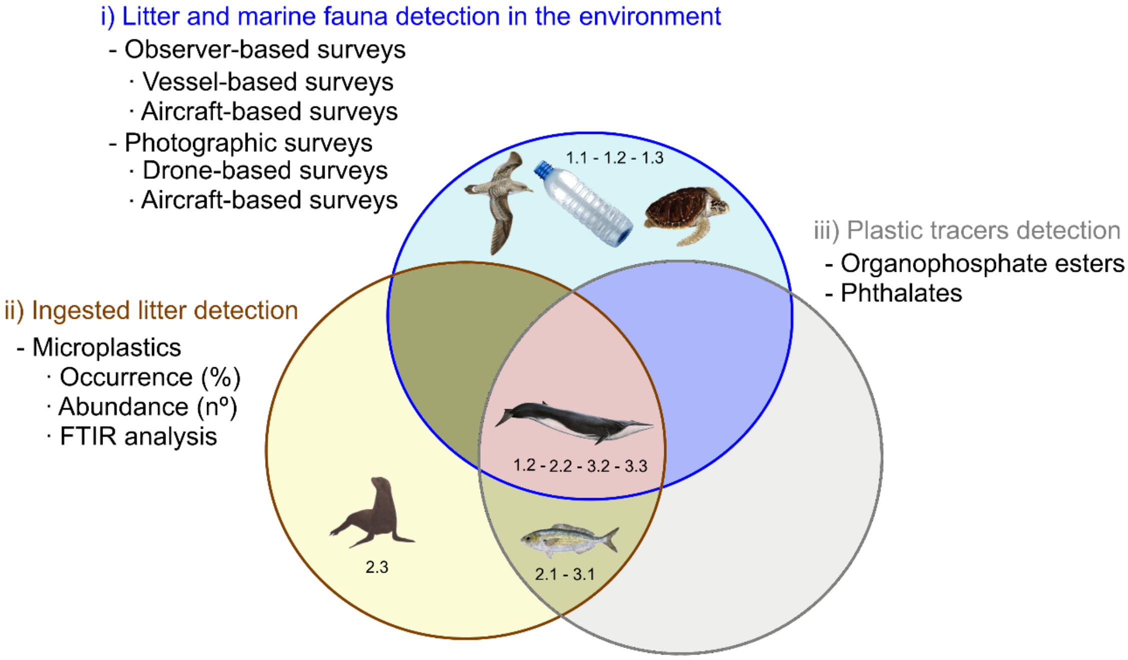
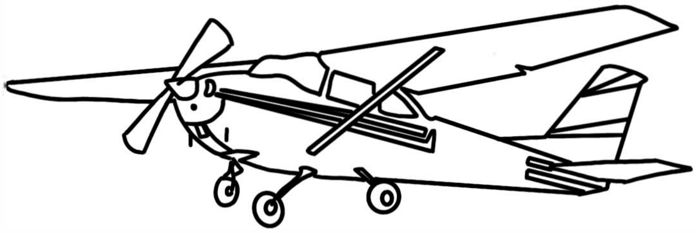


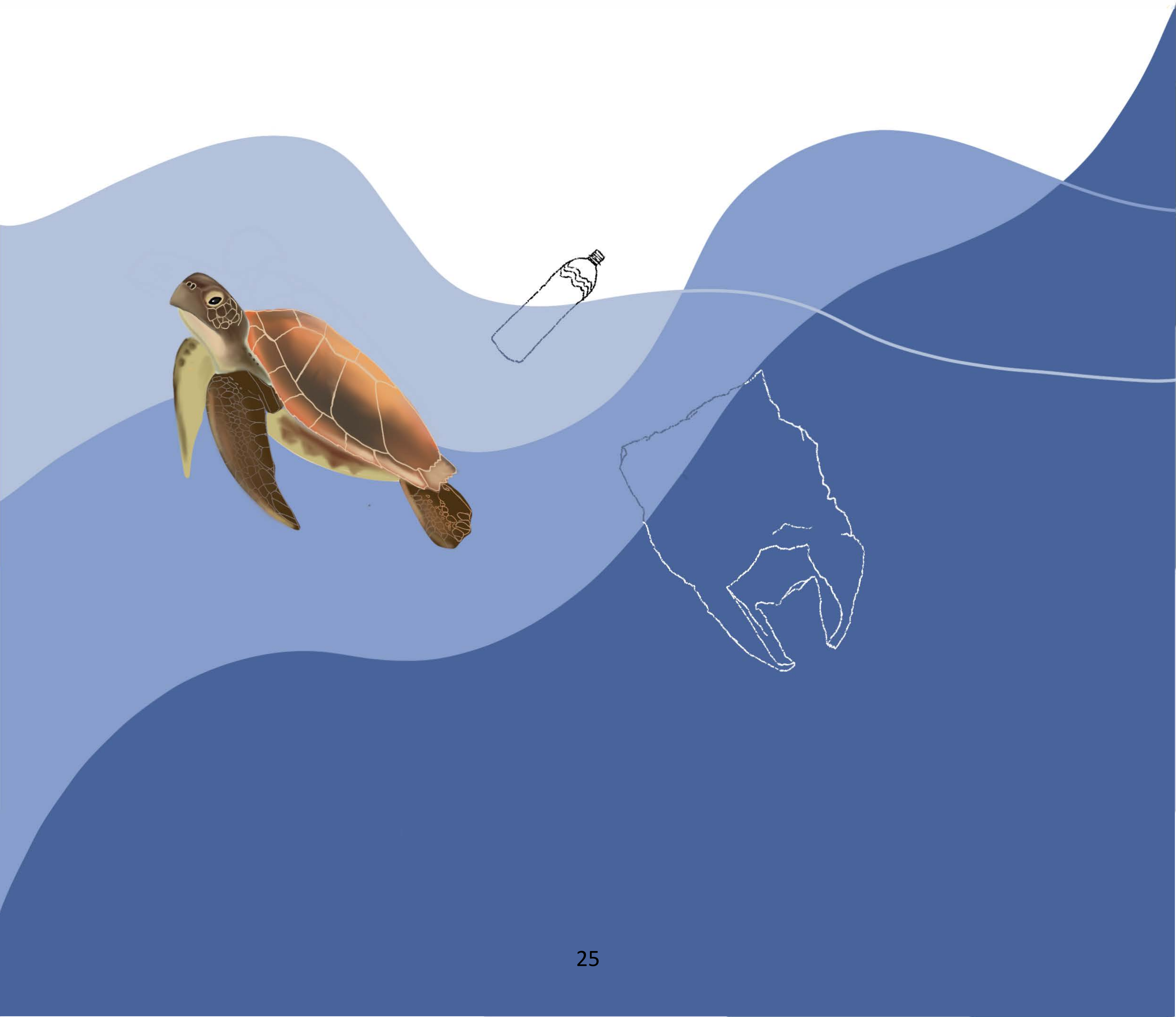
Figura 9: Triple enfoque de monitoreo para detectar la presencia de basura marina y su impacto en organismos bioindicadores. La numeración hace referencia a los diferentes subcapítulos de la presente tesis. Figura adaptada de Fossi et al. (2018).

2. **Microplásticos:** determinar la distribución, composición y abundancia de microplásticos en tres casos de estudio (diferentes áreas y especies).
    2.1. **Boga (*Boops boops*):** utilizar la boga como especie bioindicadora de microplásticos a lo largo de la costa catalana (Capítulo 2.1).
    2.2. **Rorcual común (*Balaenoptera physalus*):** utilizar el rorcual común como especie bioindicadora de microplásticos en las aguas de Islandia y al mismo tiempo determinar sus impacto en la población (Capítulo 2.2).
    2.3. **Lobo marino de dos pelos antártico (*Arctocephalus gazella*):** utilizar el lobo marino antártico como especie bioindicadora de microplásticos en las aguas del Estrecho de Bransfield (Antártida) (Capítulo 2.3).
3. **Aditivos plásticos:** estudiar la ocurrencia, composición y concentración de diferentes aditivos plásticos y su potencial impacto en dos especies de vertebrados marinos.
    3.1. **Boga**: analizar contaminantes organofosforados (aditivos plásticos) en el tejido muscular de la boga y determinar su posible relación con la ingestión de microplásticos (Capítulo 3.1).
    3.2. **Rorcual común**:
        3.2.1. Analizar contaminantes organofosforados en tejido muscular de rorcuales comunes y en su contenido estomacal (krill) para investigar su potencial bioacumulación o biomagnificación a lo largo de la cadena trófica (Capítulo 3.2).

3.2.2. Analizar contaminantes ftalatos en tejido muscular de rorcuales comunes para investigar diferencias individuales y tendencias temporales (Capítulo 3.3).



1. MACRO-RESIDUOS FLOTANTES



1.1. Floating marine macro-litter in the North Western Mediterranean Sea: Results from a combined monitoring approach

Autores: Odei Garcia-Garin[1], Asunción Borrell[1], Alex Aguilar[1], Luis Cardona[1], Morgana Vighi[1]

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

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Abstract:
The aim of the present study was twofold: (i) to validate the drone methodology for floating marine macro-litter (FMML) monitoring, by comparing the results obtained through concurrent drone surveys and visual observations from vessels, and (ii) to assess FMML densities along the North Western Mediterranean Sea using the validated drone surveys. The comparison between monitoring techniques was performed based on 18 concurrent drone/vessel transects. Similar densities of FMML were detected through the two methods (16 items km−2 from the drone method *vs* 19 items km−2 from the vessel-based visual method). The assessment of FMML densities was done using 40 additional drone transects performed over the waters off the Catalan coast. The densities of FMML observed ranged 0–200 items km−2. These results provide a validation of the use of drones to monitor FMML and contribute to increasing the knowledge about the density of FMML in the North Western Mediterranean Sea.

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Floating marine macro-litter in the North Western Mediterranean Sea: Results from a combined monitoring approach

Odei Garcia-Garin*, Asunción Borrell, Alex Aguilar, Luis Cardona, Morgana Vighi

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

A R T I C L E I N F O

Keywords:
Unmanned aerial vehicles
Drones
FMML
Remote sensing
Visual surveys

A B S T R A C T

The aim of the present study was twofold: (i) to validate the drone methodology for floating marine macro-litter (FMML) monitoring, by comparing the results obtained through concurrent drone surveys and visual observations from vessels, and (ii) to assess FMML densities along the North Western Mediterranean Sea using the validated drone surveys. The comparison between monitoring techniques was performed based on 18 concurrent drone/vessel transects. Similar densities of FMML were detected through the two methods (16 items km^{-2} from the drone method *vs* 19 items km^{-2} from the vessel-based visual method). The assessment of FMML densities was done using 40 additional drone transects performed over the waters off the Catalan coast. The densities of FMML observed ranged 0–200 items km^{-2}. These results provide a validation of the use of drones to monitor FMML and contribute to increasing the knowledge about the density of FMML in the North Western Mediterranean Sea.

1. Introduction

The worldwide production and use of plastic have extremely increased within the last decade (PlasticEurope, 2016), generating a huge amount of plastic waste. Plastic residuals are often mismanaged (UNEP, 2016), ending up in the marine environment, where plastics may account for up to 95% of the marine litter accumulated on shorelines, sea floor and sea surface (Galgani et al., 2015; UNEP/MAP, 2015). Floating marine macro-litter (FMML, *i.e.*, items > 2.5 cm (Galgani et al., 2013)) is constantly accumulating in the five subtropical gyres, where the largest densities of FMML of all oceans have been recorded (Cózar et al., 2014), but coastal areas (Ryan, 2014) and enclosed basins, such as the Mediterranean Sea, are the most affected by marine litter pollution worldwide (Cózar et al., 2015; Lambert et al., 2020; Suaria and Aliani, 2014). Indeed, densities up to 600 FMML items km^{-2} (UNEP/MAP, 2015), and 11.5 million FMML items (Lambert et al., 2020) have been detected on the surface of the Mediterranean Sea. Such levels of pollution may pose a threat to marine habitats and fauna, particularly to already endangered species like marine mammals (*e.g.*, De Stephanis et al., 2013; Garcia-Garin et al., 2020c), birds (*e.g.*, Van Franeker et al., 2011) marine turtles (*e.g.*, Digka et al., 2020; Domènech et al., 2019) and fish (*e.g.*, Romeo et al., 2015; Garcia-Garin et al., 2020d; Tsangaris et al., 2020). Hence, improving the knowledge on current levels of marine litter pollution and the support to FMML monitoring has become a critical issue (Galgani, 2019).

A number of local, European and international legislative frameworks tackling marine litter are currently in place, strongly recommending an increased effort towards FMML monitoring (*e.g.*, UNEP/MAP, Galgani et al., 2013). Within the European waters, the Marine Strategy Framework Directive (MSFD), aimed to assess the advance towards the accomplishment of the Good Environmental Status (GES), requires member states to ensure that *the composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment* (D10C1) (MSFD, 2011). The crucial importance of monitoring marine litter, and particularly its floating component, is highlighted as the first step needed to define the current level of this and other descriptors, and to plan adequate measures to achieve and maintain the GES.

Bioindicator species (*e.g.*, Domènech et al., 2019; Garcia-Garin et al., 2019, 2020b), manta trawl nets (*e.g.*, Suaria et al., 2020), manned aircrafts (*e.g.*, Garcia-Garin et al., 2020a), sailing vessels (*e.g.*, Di-Méglio and Campana, 2017) and ferries (*e.g.*, Arcangeli et al., 2018) are the most common methods used to monitor marine litter. However, the development of new remote-sensing technologies, such as satellites (*e.g.*, Topouzelis et al., 2019) and unmanned aerial vehicles (hereafter drones) (*e.g.*, Brooke et al., 2015), has opened new horizons for FMML monitoring. Although drones proved to be a cost-effective and efficient

* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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sampling method for routine beach litter (Andriolo et al., 2020; Deidun et al., 2018; Fallati et al., 2019; Gonçalves et al., 2020a; Lo et al., 2020; Merlino et al., 2020; Moy et al., 2018) and riverine monitoring (Geraeds et al., 2019), their potential to be used for marine environment monitoring is still under assessment, due to limitations mainly related with the variability of the sea surface, whose colour changes according to weather conditions, depth, type of seafloor, turbidity, cloud covering, time of day and season (Colefax et al., 2017). Thus, before this method can be properly implemented on the field, datasets obtained through its application should be validated, to guarantee that they are comparable and consistent with those obtained through the application of existing methods.

The aim of the present study was twofold: (i) to validate the methodology for FMML monitoring based on drones through the concurrent application of drone monitoring and vessel-based visual observations (hereafter referred as drone and vessel methods), and the comparison of the FMML densities obtained; and (ii) to assess the variations in FMML density across anthropized areas and Marine Protected Areas (MPAs) along the North Western Mediterranean Sea, using the previously validated drone method.

2. Materials and methods

2.1. Study area

The drone and vessel surveys were performed off the coast of Catalonia (North Western Mediterranean Sea), in an area located between the Delta de l'Ebre MPA and the Cap de Creus MPA (Fig. 1A). The drone surveys were conducted in the Cap de Creus MPA, Delta de l'Ebre MPA, and the waters around the city of Barcelona during June 2018, February 2019, and May 2019, respectively. The concurrent drone and vessel validation experiment took place between June and July 2019 in the waters off Barcelona. As the effect of wind on the sea surface (measured through the Beaufort scale) can affect the detectability of objects at sea, all surveys were conducted in conditions of low wind

Table 1
Details of the transects performed for the validation experiment and for the FMML assessment.

	Validation experiment		FMML assessment
	Drone surveys	Vessel surveys	Drone surveys
Number of images	1300	–	6300
Number of transects	18	18	58
Altitude (m)	65	2.5	45–65
Ground sampling distance (cm pixel^{-1})	2	–	2
Length of the surveyed area (km)	22.9	85	36.1
Transect width (m)	80–280	10	80–510
Surveyed area (km^2)	4.3	0.9	12.0

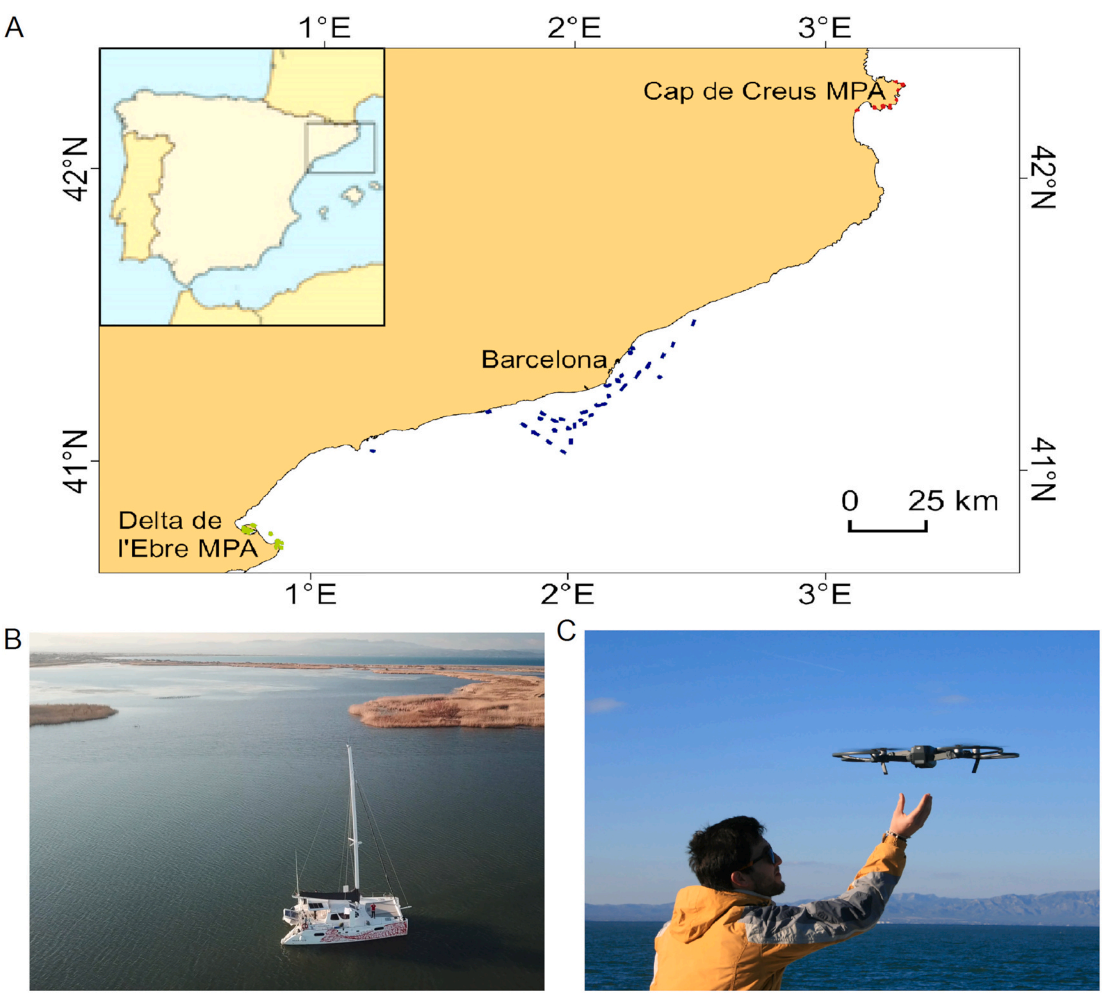

Fig. 1. Study area and GPS tracks of the drone transects at Delta de l'Ebre MPA (marked in green), Barcelona (in blue) and Cap de Creus MPA (in red) (A); snapshots of the sailing catamaran used as platform for the field surveys (B), and the DJI Mavic Pro drone take off (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

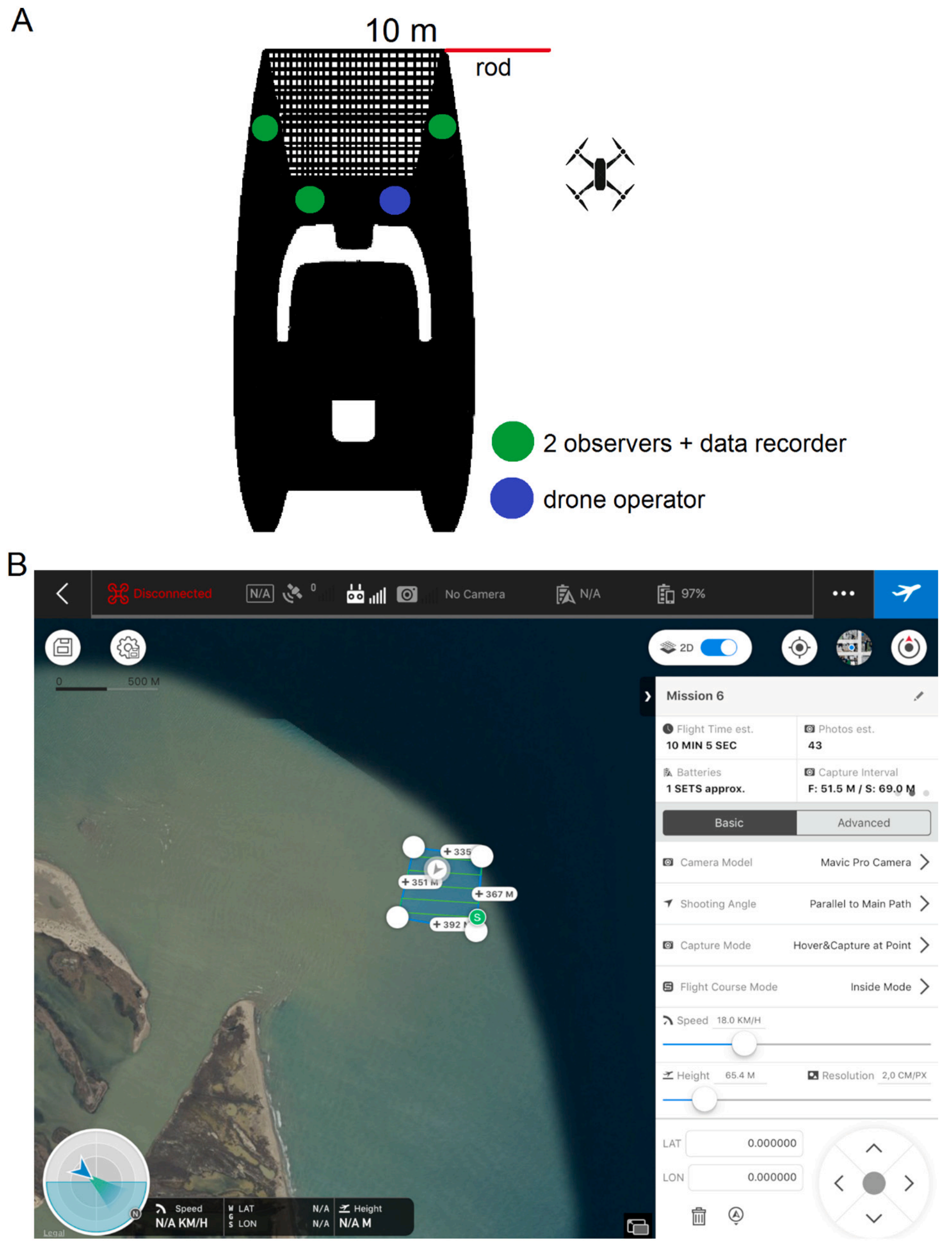

Fig. 2. Scheme of the experiment with concurrent vessel and drone transects performed from the sailing catamaran (A) and example of a drone flight plan for a transect at the Delta de l'Ebre MPA (B).

force and calm sea (*i.e.*, Beaufort sea state < 3). Water colour, turbidity and cloud shadows are also liable to affect litter detectability. However, as monitoring was conducted simultaneously with the two methods, these parameters had no effect on the results of our experiment.

2.2. Validation experiment

A total of 18 concurrent vessel and drone transects were conducted over the waters off Barcelona (Table 1). A sailing catamaran (14 m length * 7 m beam; Fig. 1B) was used as platform both for visual observations and as platform to fly and land the drone (Fig. 1C).

The standard team for the vessel survey included two trained observers, one at each side of the vessel, and a person in charge of recording the information collected by the observers (Fig. 2A). The FMML was monitored in standard conditions at a constant speed of 5 knots, within a fixed strip of 10 m delimited by the 7 m beam of the catamaran and a 3 m rod attached at one side in front of the vessel. Only those objects observed within the strip were recorded (details of the protocol used are provided in MEDSEALITTER consortium (2019)) (Table 1). When the observers reported a floating item, the data recorder took note of the time, the GPS position, and the category and number of FMML items (Table S1).

To obtain results comparable with the drone method, only the FMML items larger than 20 cm were included in the analyses, which corresponded to the 15.4% of the total FMML detected by the vessel method.

See Table 1 for the details of the transects performed for the comparison between methods, Section 2.3 and Fig. 2B for the design of the drone transects and Section 2.4 for the statistical treatment of data.

2.3. Drone surveys

A total of 58 drone transects were conducted over the waters of the Catalan coast (Fig. 1A): 18 concurrent vessel and drone transects were performed for the validation of the drone method, and 40 transects were performed to obtain the images used for the assessment of FMML

Table 2
Results from the comparison between the drone and vessel methods.

	Vessel method	Drone method
Number of FMML items detected	17	70
Density (items km^{-2}; mean ± SD)	19.7 ± 25.8	16.4 ± 21.7
Range (items km^{-2})	0–70.3	0–81.5
Plastic items (%)	100	98

density (Table 1). Of the latter, 15 took place in the Delta de l'Ebre MPA, 16 over the waters off Barcelona, and 9 in the Cap de Creus MPA (Table S2). A total of 6300 images were acquired, 1300 of which were used for the comparison of methods.

Mid-range commercial drones, namely, a Phantom 3 Pro (specification: https://www.dji.com/es/phantom-3-pro) and a DJI Mavic Pro (specification: https://www.dji.com/mavic; Fig. 1C), equipped with a 12 megapixels camera, were used to perform this study. The DJI Ground Station Pro and Pix4D applications were used to control the drones from a tablet, allowing automatic flights for scanning specific areas (Fig. 2B). Flight height was set to 45 m when using the Phantom 3 Pro and 65 m when using the DJI Mavic Pro, to guarantee a ground sampling distance of 2 cm $pixel^{-1}$, which only allows the detection of FMML items larger than 20 cm, but guarantee covering a large area (details of the protocol used are provided in MEDSEALITTER consortium (2019)). The camera was pointed at nadir (90° to the ground) with automatic settings and was connected to the GPS signal of the drone. Each image spanned 4000 × 3000 pixels.

Although it is recommended that at least three researchers inspect images separately, given the high number of images obtained here, only one experienced photo-interpreter could review the images to detect and identify the targets. However, the photo-interpreter was familiar with the kind of items that are abundant on the Catalan coast, and was also involved in all field campaigns. Doubtful FMML items were checked by a second researcher to avoid potential errors as in Garcia-Garin et al. (2020a). Items detected in consecutive images were carefully compared to avoid to mark the same item twice. The average time dedicated to inspecting each image was 20 s, leading to an overall effort of approximately 35 h for the analysis of all the images.

2.4. *Classification of detected targets*

FMML items were classified by category and composition according to the master list for FMML proposed by the Technical Subgroup on Marine Litter within the Marine Strategy Framework Directive (Galgani et al., 2013), which was revised according to the MEDSEALITTER project (MEDSEALITTER consortium, 2019; Table S1).

2.5. *Statistical analysis*

The sampling units used to analyse the observations obtained from the drone surveys were created by grouping all the images taken during a given transect. The sampling units used to analyse the observations obtained from the vessel surveys were created by grouping all FMML sightings taking place in each adjacent area of a given drone transect, keeping a constant ratio of 5:1 between the drone area and the vessel area. This ratio was the most suitable to compare adjacent samples between methods, as the surveyed area by the drone method was five times higher than that by the vessel method (Table 1).

2.5.1. *Validation experiment*

Prior to analysis, data were tested for normality and homogeneity of variance using Shapiro-Wilk test and Levene's test, respectively. Whenever the tests showed that data distribution departed from homogeneity of variance or normality, FMML densities of concurrent vessel and drone surveys were compared across the sampling units using the non-parametric paired-sample Wilcoxon signed-rank test.

The categories of FMML items observed with the two methods were compared using the Pearson's Chi-squared test.

2.5.2. *FMML density assessment*

FMML density was modelled using GLMs (generalized linear models) fitted with a negative binomial error distribution to account for overdispersion. GLMs were used to assess the relative effect on FMML density of the sampling area (categorized as: Delta de l'Ebre MPA, Barcelona and Cap de Creus MPA), and the distance to the coastline, calculated for each sampling unit using the measuring tool in Qgis (QGIS Development Team, 2018). The information-theoretic approach was used for model selection (Burnham and Anderson, 2002) and models were compared using the AIC (Akaike's Information Criterion) (Akaike, 1974). A Tukey HSD test was performed to compare FMML densities in the three sampling areas.

The level of significance was set at $p < 0.05$. Analyses were conducted using R (R Core Team, 2018).

3. Results

3.1. *Validation experiment*

A total of 17 FMML items were detected through the vessel method in the 0.9 km^2 surveyed area (Table 2). Plastic sheets, fishing nets and bags accounted for the 89% of the total FMML detected (Fig. 3A). A total of 70 FMML items were detected through the drone method in the 4.3 km^2 survey area (Table 2). Unidentified plastics, bags and bottles accounted for the 89% of the total (Fig. 3B). The FMML density detected in each sampling unit is shown in Table S2.

FMML densities detected with the two methods were compared *via* a paired-sample Wilcoxon's signed-rank test. The median of the differences between the vessel and the drone methods across the sampling units was not significantly different from zero ($p = 0.9$; Fig. 4). However, the proportion of litter categories identified by the two methods differed (Pearson's Chi-squared test, $p < 0.001$), being sheets and unidentified plastics the most dissimilar.

3.2. *FMML density assessment*

For the assessment of FMML density, the 6300 images acquired throughout the totality of the drone surveys were considered. The number, mean density and density range of FMML items detected in the three study areas are shown in Tables 3 and S2. Most of the FMML items detected were made of plastic (99%), of which 78% were unidentified fragments, 8% bags and 7% aggregations (Fig. 3C). Densities of FMML in the three sampling areas are shown in Fig. 5. Examples of aerial images of FMML taken by the drone are shown in Fig. 6.

Four different GLMs were fitted taking into consideration 2 variables (distance to the coastline and area), their combination and their interaction (Table 4). The model with the lowest AIC score was that including the interaction between the area and the distance to the coastline (M1, AIC = 456; Table 4), suggesting that higher densities of FMML occur in sampling sites closer to the coast, in the waters of the Delta de l'Ebre MPA (Tables S3, S4).

4. Discussion

In this study, we first aimed to validate the use of drones for FMML monitoring through a concurrent vessel and drone monitoring experiment, which showed that the two methods produce comparable results. Subsequently, we applied the drone method to monitor FMML along the Catalan coast, where we detected densities of FMML ranging from 0 to 200 items km^{-2}, with the highest mean values in the Delta de l'Ebre MPA.

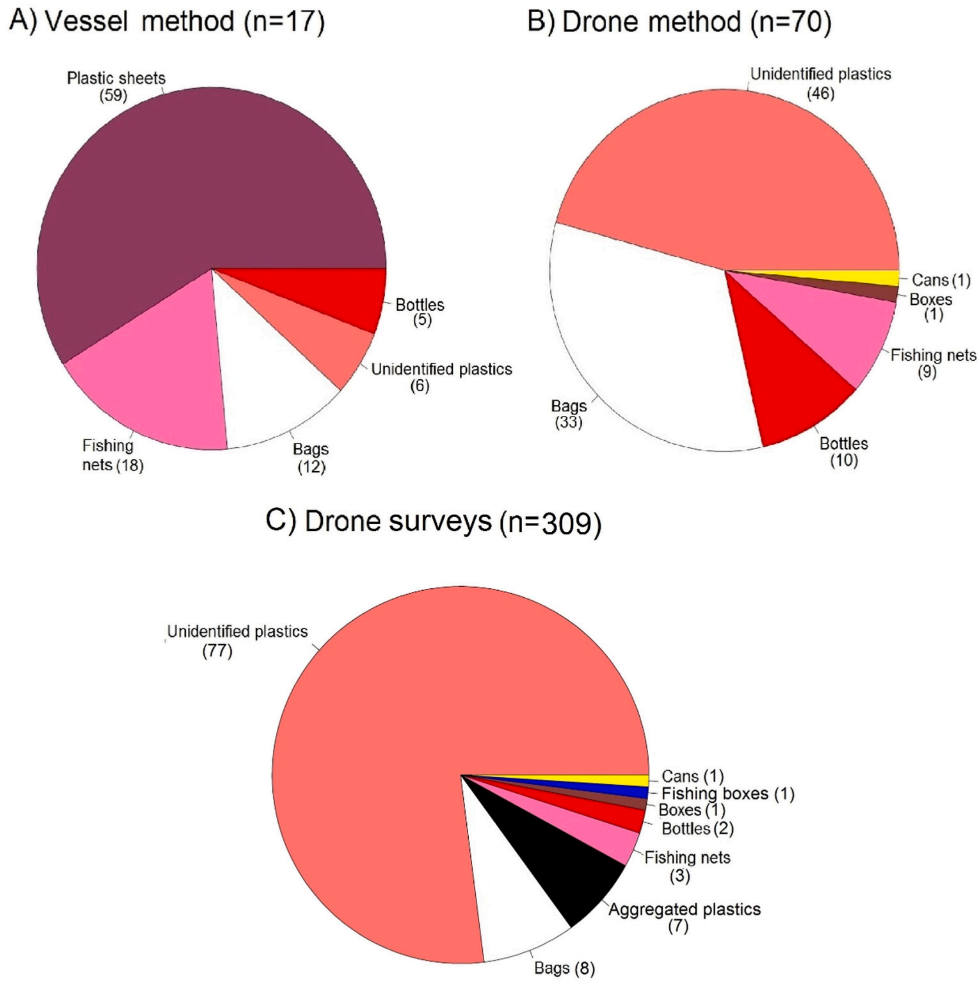

Fig. 3. Proportions of the different FMML types detected during the validation experiment, from the vessel method (A) and the drone method (B); and FMML detected throughout the totality of drone surveys conducted along the Catalan coast (C) (n = total FMML detected). The percentage of items detected for each FMML category is represented in brackets.

4.1. Validation experiment

A recent report from the European Commission (European Commission, 2017) stated that almost all Member States have monitoring programmes in place for beach litter monitoring, and high advances are done in automated drone monitoring for this marine compartment (Gonçalves et al., 2020a, 2020b). However, there are still gaps related to the application of this technique for floating litter monitoring (Deidun et al., 2018; European Commission, 2017).

The drone method presents valuable benefits over traditional observer-based monitoring techniques: (i) images can be re-analysed several times by multiple photo-interpreters and/or using machine learning techniques, reducing the human error involved with the detection and identification of items; (ii) the survey area and the size of the detected objects can be precisely determined if the ground sampling distance of the image is known; and (iii) images can be used by researchers working on more than one subject, potentially reducing costs and human resource needs. However, mid-range commercial drones also present some limitations, including (i) low flight endurance (10–20 min), in turn limiting the spatial coverage of monitoring; (ii) possible flight constraints imposed by local legislation (data protection regulations, for instance, imposed flight restrictions in many countries); and (iii) the difficulty of detecting and quantifying marine litter on aerial images due to the high variability of materials, items, colours, and also of environmental conditions (Colefax et al., 2017; Deidun et al., 2018; Fiori et al., 2017; Gonçalves et al., 2020b).

Our results showed that FMML densities obtained through the two methods were comparable, confirming that drones operated from vessels are a proper tool to monitor FMML. However, the proportions of the items' categories detected were significantly different. Such difference was mainly related to a higher proportion of sheets detected by the vessel method, and a higher proportion of unidentified plastic items detected by the drone method, which might be a consequence of the higher altitude of monitoring, and thus a lower precision, of the drone method with respect to the vessel method. Apparently, the human eye could better determine the item category when observing it 'live' from the vessel, than when analysing the aerial images. Thus, the category of many items that resembled plastics (46% of the total) could not be identified in the images obtained with the drone method. Although the vessel method allowed monitoring a smaller area than the drone method, by extrapolating the results obtained from the vessel method, we may assume that most of the unidentified plastics detected by the drone method could be plastic sheets. Furthermore, it should be noted that the drone method only allowed the detection of items larger than 20 cm, which represented only one sixth of the total FMML detected through the vessel method.

To cope with these biases, one solution might be to reduce the drone flight height to increase the image resolution and reduce the minimum size of items detectability. However, while aerial imagery collected at low altitudes has a very high resolution, it covers only a relatively small area (Deidun et al., 2018). For the present study, flight altitude was set to guarantee a balance between the resolution of images and the

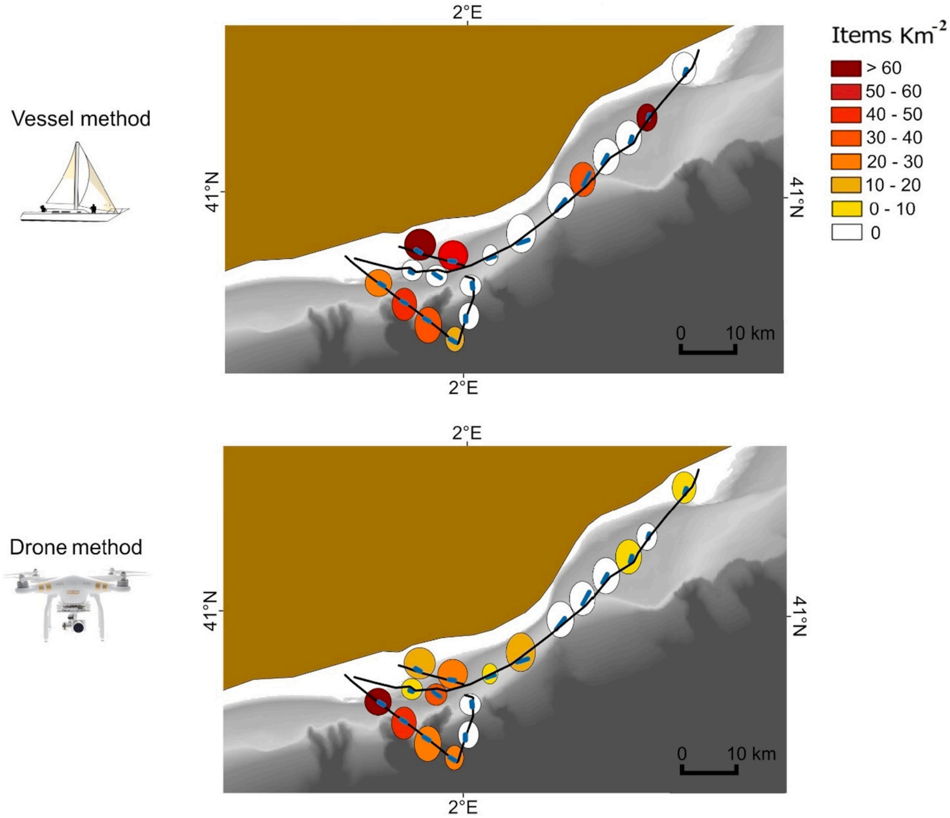

Fig. 4. Densities of FMML detected through the vessel method (black continuous lines) and the drone method (blue segments of lines). Circles represent the sampling units considered for each method. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

covered area, which had to be large enough to perform statistical analysis (MEDSEALITTER consortium, 2019).

To further improve the drone method, the FMML detection process in images should be automated to reduce the time required for image processing. Indeed, some researchers have developed new algorithms for FMML detection, such as Kylili et al. (2019), who used a deep learning algorithm to detect marine litter at very low distance, or Goddijn-Murphy et al. (2018), who developed a hyperspectral algorithm. However, these algorithms are still not applicable for FMML monitoring from long distances at sea, where no spatial references are present and sun glint and waves have an adverse impact on detectability (Colefax et al., 2017).

4.2. FMML density assessment

The densities of FMML observed in the Catalan coast through the drone surveys are consistent with those reported for items larger than 2 cm in the central-western Mediterranean Sea (24.9 items km^{-2}; Suaria and Aliani, 2014). Scaled up (*i.e.*, taking into account that drone surveys can detect from one fourth to one sixth of the total FMML), densities detected in the present study would be from four to six times higher than those reported by Suaria and Aliani (2014), and one order of magnitude higher than that observed from ferries in the same area and the same size range (2.5 items km^{-2}; Arcangeli et al., 2018). However, in the latter, the high speed and height of the observer above the sea level might have decreased the detectability of FMML, reducing the mean density. A recent observer-based aerial survey, conducted over almost the entire Mediterranean Sea and including only items larger than 30 cm, reported FMML densities ranging from 0 to 5 items km^{-2} in the Western Mediterranean, and densities similar to our study in the Adriatic Sea (Lambert et al., 2020). Overall, the densities observed in the present study are higher than those reported by studies using observer-based methods in the same area (Arcangeli et al., 2018; Lambert et al., 2020; Suaria and Aliani, 2014), confirming the validity of the drone method to monitor FMML.

Our results were also consistent with other studies reporting proportions of over 80% of plastic items in FMML (*e.g.*, Arcangeli et al., 2018; Campanale et al., 2019; Galgani et al., 2015; UNEP/MAP, 2015). According to other studies and the results from our vessel surveys, the high number of unidentified and aggregated plastics could belong to the categories of plastic sheets, fragments of bags or wrappings (Arcangeli et al., 2018).

FMML densities were significantly different throughout the surveyed area. Although a higher density in the waters off Barcelona might be expected, results from the best-fit model showed that higher densities of FMML are found close to the coastline in the Delta de l'Ebre

Table 3
FMML (number of items, mean density and density range of items detected) assessed through drone surveys conducted at Delta de l'Ebre MPA, Barcelona and Cap de Creus MPA.

	Delta de l'Ebre MPA	Barcelona	Cap de Creus MPA	Total
Number of FMML items detected	102	189	18	309
Density (items km^{-2}; mean ± SD)	29.1 ± 28.1	23.9 ± 38.5	16.7 ± 22.3	24.1 ± 33.7
Range (items km^{-2})	0–76.4	0–200.6	0–67.2	0–200.6

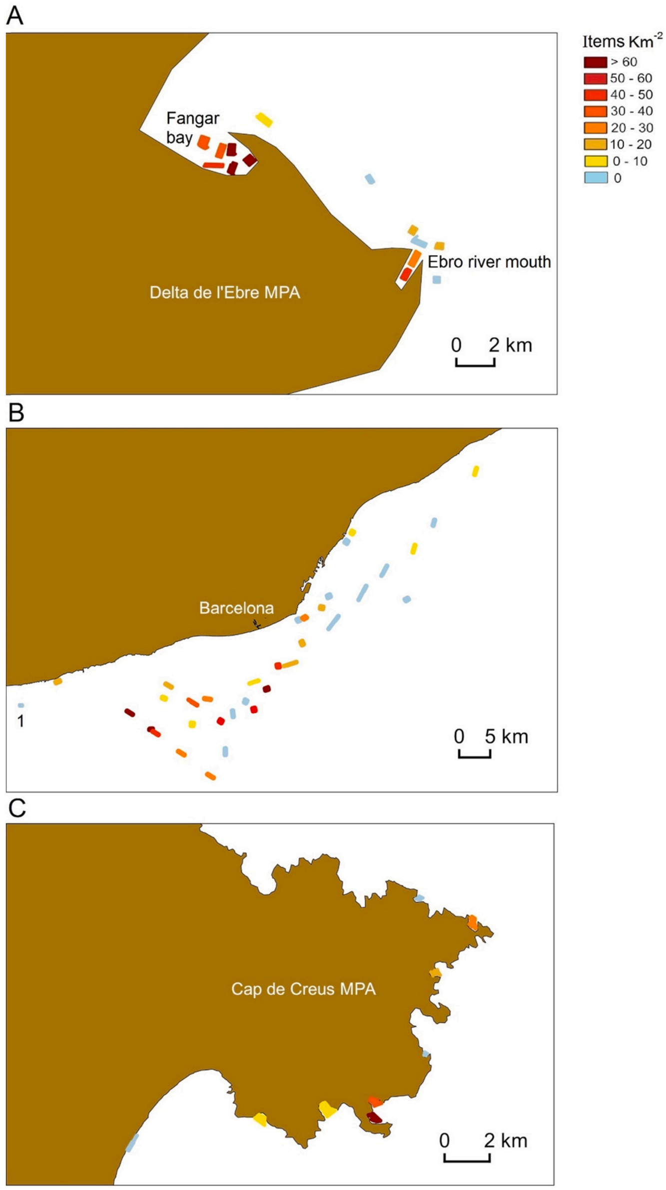

Fig. 5. Density of FMML detected in the Delta de l'Ebre MPA (A), the Barcelona area (B) and the Cap de Creus MPA (C). Transect marked as "1", which was in front of the city of Tarragona (41.05 N, 1.23 E), was moved Eastwards to improve the visibility of the other transects.

MPA. Likely, most of the FMML found in Delta de l'Ebre MPA is discharged by the Ebro river, which is the second longest and largest river in terms of flow rate of the entire Iberian Peninsula. Furthermore, the prevailing marine currents along the western coast of the Mediterranean Sea run along the continental slope from North to South (Font et al., 1995). Hence, it is likely that the FMML derived from the city of Barcelona, which is the second Mediterranean city in terms of inputs of plastic litter (*i.e.*, 1800 tons per year; Liubartseva et al., 2018), is carried by marine currents to the Delta de l'Ebre MPA. Inspecting in detail the FMML densities obtained from each drone transect in this area, the highest values are observed in the semi-enclosed Fangar bay and in the mouth of the Ebro river. Since semi-enclosed bays can accumulate high amounts of FMML, and most FMML is driven by rivers to the sea (Schirinzi et al., 2020), these results were somehow predictable. Similarly, the concentrations areas of FMML detected in the Balearic sea (Arcangeli et al., 2018; Mansui et al., 2015), where FMML is likely driven by currents and retained in the central area (Mansui et al., 2015), show densities of FMML consistent with those found off the city of Barcelona, up to 200 items km^{-2}.

No significant differences were detected between the FMML density in the Cap de Creus MPA and that found in the other two areas, although the Cap de Creus MPA showed the lowest values. Despite being protected, the Cap de Creus MPA is a common destination for local and international tourists due to its natural values, and consequently, high quantities of litter are produced on the land that might enter the sea. In addition, the main pattern of winds and currents from North to South might also generate local areas of FMML accumulation. However, it should be noted that the drone surveys in Cap de Creus MPA were conducted one year before those conducted in the other two areas, and during a different season, so a proper comparison is not feasible. Further surveys should be carried out to compare FMML densities between these areas, taking their likely seasonal variations into account.

5. Conclusions

Our results provide an experimental validation of the drone method to monitor FMML items larger than 20 cm, supporting the use of drones as cost-effective method for FMML monitoring in small scale marine areas such as MPAs and coastal areas. Furthermore, the area off the city of Barcelona was identified as highly polluted, with FMML densities up to 200 items km^{-2}, although a higher FMML mean density was reported in the area of Delta de l'Ebre MPA, highlighting that currents and river discharges may carry FMML to this less anthropized area. Further research is needed to assess the temporal variations of FMML densities along the Catalan coast, which would contribute to the assessment of marine litter trends in the Mediterranean Sea. As well, the development of a suitable algorithm for the automatic detection of FMML in the aerial images taken by drones would substantially improve the performance of this method.

CRediT authorship contribution statement

Odei Garcia-Garin: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization. **Asunción Borrell:** Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Alex Aguilar:** Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Luis Cardona:** Resources, Writing - review & editing, Funding acquisition. **Morgana Vighi:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Resources.

Declaration of competing interest

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

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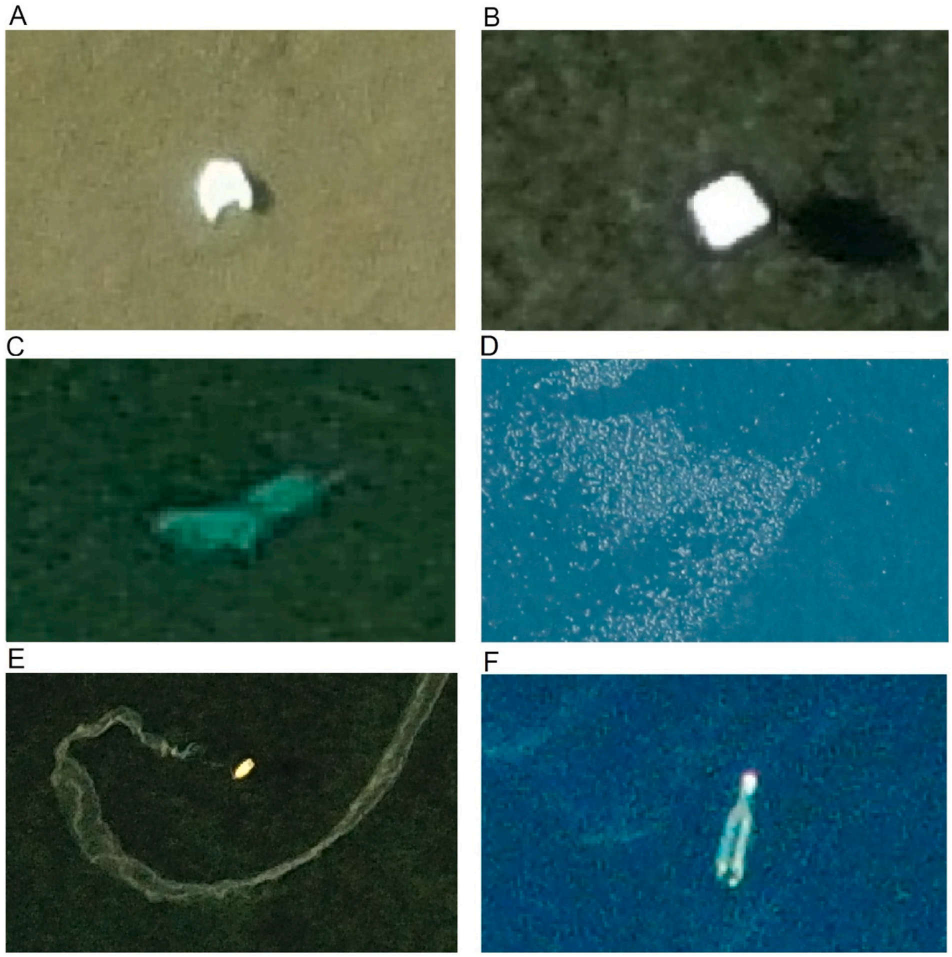

Fig. 6. Examples of FMML items ((A) plastic bag, (B) fishing box, (C) unidentified plastic, (D) aggregated plastics, (E) fishing net and (F) bottle) detected through the drone method. Images were cropped to improve the visibility of items.

Table 4
GLMs fitted with a negative binomial error distribution, ranked by Akaike Information Criterion (AIC) for FMML densities. Explanatory variables included in the models: area (categorized in: Delta de l'Ebre MPA, Barcelona and Cap de Creus MPA) and distance to the coastline (Coast, m). The best-fit model is shown in bold.

	Model	AIC
M1	**Coast *area**	**456**
M2	Coast + area	459
M3	Coast	456
M4	Area	458

improve the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111467.

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1.2. Who's better at spotting? A comparison between aerial photography and observer-based methods to monitor floating marine litter and marine mega-fauna

Autores: Odei Garcia-Garin[1], Alex Aguilar[1], Asunción Borrell[1], Patricia Gozalbes[2], Agustín Lobo[3], Jaime Penadés-Suay [2], Juan A. Raga[2], Ohiana Revuelta[2], Maria Serrano [1], Morgana Vighi[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain
[2]Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, PO Box 22085, 46071, Valencia, Spain
[3]Instituto de Ciencias de la Tierra "Jaume Almera" (CSIC), Lluis Solé Sabarís s/n, 08028, Barcelona, Spain

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Abstract:
Pollution by marine litter is raising major concerns due to its potential impact on marine biodiversity and, above all, on endangered mega-fauna species, such as cetaceans and sea turtles. The density and distribution of marine litter and mega-fauna have been traditionally monitored through observer-based methods, yet the advent of new technologies has introduced aerial photography as an alternative monitoring method. However, to integrate results produced by different monitoring techniques and consider the photographic method a viable alternative, this 'new' methodology must be validated. This study aims to compare observations obtained from the concurrent application of observer-based and photographic methods during aerial surveys. To do so, a Partenavia P-68 aircraft equipped with an RGB sensor was used to monitor the waters off the Spanish Mediterranean coast along 12 transects (941 km). Over 10000 images were collected and checked manually by a photo-interpreter to detect potential targets, which were classified as floating marine macro-litter, mega-fauna and seabirds. The two methods allowed the detection of items from the three categories and proved equally effective for the detection of cetaceans, sea turtles and large fish on the sea surface. However, the photographic method was more effective for floating litter detection and the observer-based method was more effective for seabird detection. These results provide the first validation of the use of aerial photography to monitor floating litter and mega-fauna over the marine surface.

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Who's better at spotting? A comparison between aerial photography and observer-based methods to monitor floating marine litter and marine mega-fauna☆

Odei Garcia-Garin [a, *], Alex Aguilar [a], Asunción Borrell [a], Patricia Gozalbes [b], Agustín Lobo [c], Jaime Penadés-Suay [b], Juan A. Raga [b], Ohiana Revuelta [b], Maria Serrano [a], Morgana Vighi [a]

[a] Department of Evolutionary Biology, Ecology and Environmental Sciences, Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, 08028, Barcelona, Spain
[b] Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, PO Box 22085, 46071, Valencia, Spain
[c] Instituto de Ciencias de la Tierra "Jaume Almera" (CSIC), Lluis Solé Sabarís s/n, 08028, Barcelona, Spain

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Pollution by marine litter is raising major concerns due to its potential impact on marine biodiversity and, above all, on endangered mega-fauna species, such as cetaceans and sea turtles. The density and distribution of marine litter and mega-fauna have been traditionally monitored through observer-based methods, yet the advent of new technologies has introduced aerial photography as an alternative monitoring method. However, to integrate results produced by different monitoring techniques and consider the photographic method a viable alternative, this 'new' methodology must be validated. This study aims to compare observations obtained from the concurrent application of observer-based and photographic methods during aerial surveys. To do so, a Partenavia P-68 aircraft equipped with an RGB sensor was used to monitor the waters off the Spanish Mediterranean coast along 12 transects (941 km). Over 10000 images were collected and checked manually by a photo-interpreter to detect potential targets, which were classified as floating marine macro-litter, mega-fauna and seabirds. The two methods allowed the detection of items from the three categories and proved equally effective for the detection of cetaceans, sea turtles and large fish on the sea surface. However, the photographic method was more effective for floating litter detection and the observer-based method was more effective for seabird detection. These results provide the first validation of the use of aerial photography to monitor floating litter and mega-fauna over the marine surface.

1. Introduction

Floating marine macro-litter (FMML, *i.e.*, items larger than 2.5 cm in length, Galgani et al., 2013) can cause severe injuries to marine organisms; entanglement and/or accidental ingestion has been reported in various species of marine birds (*e.g.*, Van Franeker et al., 2011), cetaceans (*e.g.*, De Stephanis et al., 2013; Di-Méglio and Campana, 2017), turtles (*e.g.*, Camedda et al., 2014; Domènech et al., 2019) and fish (Boerger et al., 2010). Due to the ever-increasing pressure from marine litter, a number of regional, national, and international legislative regulations recommend an increase in monitoring efforts and the development of efficient and standardized methods to monitor FMML and its impacts on marine organisms. The systematic collection of data on the abundance, distribution and trends of FMML and mega-fauna would contribute to the identification of potential risk areas/seasons and to a better assessment of the magnitude of this threat.

FMML and marine fauna have been traditionally monitored through observer-based methods, either applied from marine platforms such as ferries and other kinds of vessels (*e.g.*, Arcangeli et al., 2017; Di-Méglio and Campana, 2017; Fortuna et al., 2007; Suaria and Aliani, 2014) or from manned aircraft. Observer-based aerial surveys have been extensively used in terrestrial

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* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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environments and are widely used to monitor the abundance and distribution of FMML and mega-fauna in the sea (*e.g.*, Brooke et al., 2015; Gómez de Segura et al., 2007; Hodgson et al., 2013; Lecke-Mitchell and Mullin, 1997; Unger et al., 2014). However, the accuracy of the data obtained through observer-based methods may present some biases, mainly related to the experience and training of the observers (Colefax et al., 2017; McEvoy et al., 2016).

During the last decade, manned aircraft and unmanned aerial vehicles (UAVs) equipped with different types of cameras have been widely employed to monitor marine fauna worldwide, including seabirds (Büttger et al., 2015), sea turtles (Gordon et al., 2013), harbour seals (Hoeschle et al., 2015), harbour porpoises (Williamson et al., 2016), dugongs (Hodgson et al., 2010), and several other cetacean species (Gibbs et al., 2019). In addition, infrared cameras, RGB video cameras and LIDAR installed in manned aircraft have allowed the detection and monitoring of, among other things, derelict nets in the Gulf of Alaska (Pichel et al., 2012), FMML within the "Great Pacific Garbage Patch" (Garaba et al., 2018; Gibbs et al., 2019; Lebreton et al., 2018), macro-litter on beaches (Nakashima et al., 2011) and oil spills (*e.g.,* Bradford and Sanchez-Reyes, 2011; Leifer et al., 2012). In addition, satellite imagery will also represent a useful tool for monitoring the sea surface in the near future (Cubaynes et al., 2018; Topouzelis et al., 2019).

However, the areas covered by photographic surveys are generally smaller than those that could be covered by observer-based surveys performed using distance sampling methods (Buckland et al., 2001; Buckland et al., 2015), which have no limitations related to the storage space or battery charge duration of the recording systems. Moreover, despite the wide use of aerial photography for monitoring purposes and the great efforts to develop suitable algorithms for the analysis of the very large number of images obtained (Goddijn-Murphy et al., 2018; Kylili et al., 2019), the currently available algorithms for the automated detection and identification of FMML in aerial images are still far from perfect, and the analyses are still often performed manually.

However, despite the disadvantages presented above, the use of aerial photography provides major benefits over traditional observer-based surveys, including 1) an increase in accuracy, as the survey area can be precisely designated *a priori* or determined *a posteriori* from the images, and the exact size of the targets can be calculated when the image ground sampling distance (GSD) is known; 2) a reduction in human error, as the images provide a permanent record, which allows subsequent re-analysis by multiple photo-interpreters to check doubtful targets and to answer further scientific questions; and 3) a reduction in human safety risks and costs, because during photographic surveys, only the pilot and possibly a camera operator have to board the aircraft; the trained personnel time is reduced, and the processing time could be further reduced by applying automated algorithms (Thaxter and Burton, 2009).

For these reasons, aerial photography methods are likely to detect higher densities of FMML and mega-fauna and are increasingly used for monitoring programmes. However, as most of the available data and information included in the baseline studies have been collected through observer-based surveys; to allow the integration of data obtained through photographic methods into existing observer-based databases, it is essential to test whether the results obtained from these two methodologies are comparable.

The aim of this study was to compare the FMML and marine mega-fauna observations produced by concurrent observer-based and photographic aerial surveys to validate the use of aerial photography to monitor the marine surface. The results of such a validation would allow a step forward in the assessment of the long-term trends of the FMML distribution and its potential impacts on marine biodiversity.

2. Materials and methods

2.1. Flight planning

The concurrent observer-based and RGB photographic aerial surveys were performed from a high-wing aircraft (Partenavia P-68) equipped with bubble windows over the waters off the Mediterranean coast of Spain. The surveys took place in an area located between the Ebro River Delta and the province of Alicante, with depths ranging from 10 to 1300 m (Fig. 1). The flights were performed at a constant groundspeed of 90 knots (166 km h^{-1}) and a constant altitude of 230 m (750 ft), which is the minimum flight altitude based on local legislation and allows the detection of objects larger than 30 cm (Gómez de Segura et al., 2006; MEDSEALITTER consortium, 2019). A large number of seabird species (*e.g.*, the Balearic shearwater and the European storm petrel), when floating on the sea surface, are smaller than 30 cm, which is the smallest detectable size for the two methods in this study and could lead to an underestimation of relatively small birds. The surveys were conducted over 4 days in March 2018 along 12 transects. Three groups of transects (1:4, 5:8, and 9:11), established during three full working days, were equidistant and perpendicular to the coast, while transect 12 was partially parallel to the coast to guarantee suitable monitoring of the Ebro River Delta and its possible effects on FMML accumulation (Fig. 1, Table S2). The FMML, mega-fauna and seabirds were surveyed throughout all the transects, except for transect 12, on which seabirds were not considered.

2.2. Observer-based survey

The standard team for the observer-based survey included two experienced observers, one at each side of the aircraft, and a person in charge of recording the information collected by the observers. The FMML was monitored within two fixed strips of 274 m, one for each side of the aircraft. Only those objects within the strips were recorded, and the observations from the two strips were merged together for analysis (MEDSEALITTER consortium, 2019) (Table 1). The strip width was estimated using a hand-held inclinometer by considering the area between 90° and 40° (the observable area within 274 m from the transect line at an altitude of 230 m). Coloured tape marks were placed on the windows to delimit the area of observation. For the mega-fauna observations, the distance sampling method (Buckland et al., 2001) was used: the angle between the horizon and the observed individual was determined using a hand-held clinometer to estimate its perpendicular distance. However, only the mega-fauna sightings recorded within 90° and 40° on the two sides of the aircraft were included in the analyses to obtain comparable density results.

When the observers reported a sighting, the data recorder took note of the time, the position (obtained with a GPS), and either the category and number of FMML items or, in the case of marine mega-fauna sightings, the species, number of individuals and the angle of observation. The environmental conditions, including the Beaufort sea state, amount of sun glare (categorized as 0 (0–25%), 1 (25–50%), 2 (50–75%) and 3 (75–100%)), and cloud cover were also updated at the beginning of each transect and whenever any change occurred.

2.3. Photographic survey

The camera used for the photographic survey was a Canon EOS REBEL SL1, placed under the aircraft in the nadir position. The camera, connected to the GPS signal of the aircraft, was set to take a picture every 2 s, with fixed settings: 5.6 focal length, 800 ISO and

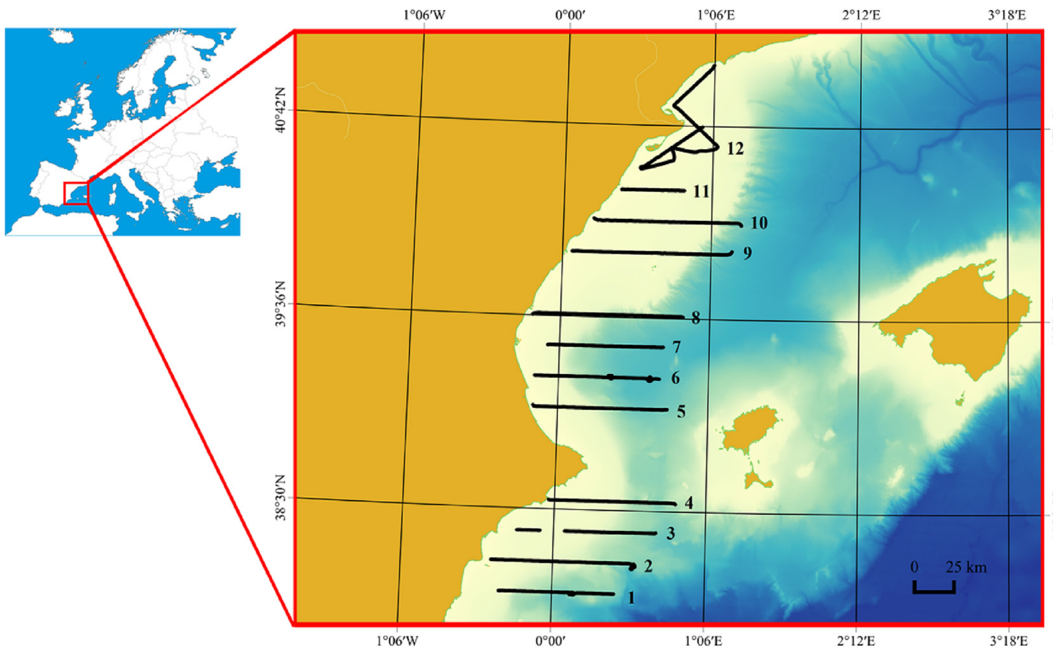

Fig. 1. Study area and GPS tracks of the surveyed transects.

Table 1
Details of the photographic and observer-based aerial surveys.

	Photographic survey	Observer-based survey
Altitude (m)	230	230
Speed (knots)	90	90
Sampling unit length (equivalent to 100 images, km)	8.6	9.3
Distance between images (m)	7.6	–
Length of the surveyed area (km)	865.2	941.1
Transect width (m)	128.2	548
Survey area (km^2)	110.9	515.7

1/2000 s shutter speed. The image footprint (1A and 1B) and GSD (2) were calculated as follows:

$$Across - track\ footprint = \frac{Flying\ height(mm)}{Focal\ lenght(mm)} *(Sensor\ width\ (pixels)*Pixel\ size\ (mm)) \tag{1A}$$

$$Along - track\ footprint = \frac{Flying\ height(mm)}{Focal\ lenght(mm)} *(Sensor\ height\ (pixels)*Pixel\ size\ (mm)) \tag{1B}$$

$$GSD = \frac{Sensor\ width\ (mm)*Flying\ height\ (m)*100}{Focal\ lenght\ (mm)*Sensor\ width\ (pixels)} \tag{2}$$

Onboard the aircraft, a person was in charge of operating the camera from a tablet through the Waldo Flight Control System software. As images were taken every 2 s at a speed of 90 knots and an altitude of 230 m, there was a gap of 7.6 m between consecutive images. Consequently, the area covered by the photographic transects was smaller than that covered by the visual transects, which was accounted for in the density calculations (Table 1).

To reduce error, it is recommended that at least three researchers inspect images separately, and if the three detection estimates differ by less than 10%, the final estimate is calculated as the arithmetic mean of the three values. However, given the high number of images obtained in the present study, only one experienced photo-interpreter manually reviewed the images to detect and identify the targets. Doubtful target identifications were checked by a second researcher to confirm potential detections. The average time dedicated to the visual analysis of each image was 20 s, leading to an overall effort of approximately 56 h for the inspection of all the images.

2.4. Classification of the detected targets

The targets detected through both methods were classified into three main categories: FMML, mega-fauna (*i.e.*, cetaceans, marine turtles and sunfish) and seabirds. The seabirds were not identified to species and were analysed separately due to their different behaviour relative to other mega-fauna. Floating liquids (*e.g.*, oil and foams), organic matter and unidentified items were not included in the analysis of the FMML.

The FMML was classified by category and composition according to the master list for floating objects proposed by the Technical Subgroup on Marine Litter within the Marine Strategy Framework Directive (Galgani et al., 2013), which was modified according to the guidelines provided by the Interreg MED MEDSEALITTER project (MEDSEALITTER consortium, 2019; see Table S1).

2.5. Statistical analysis

The sampling units were created by grouping 9.3 linear km along each transect, a length encompassing 100 images. However, if the total length of the transect was not an exact multiple of 9.3 km, the excess area was grouped together with the adjacent sampling unit, and a larger sampling unit was created. Each image/observation was associated with a given sampling unit on the basis of its respective GPS coordinates. The densities of the targets detected within each sampling unit were calculated as items/km^2 (Table 1).

The normality and heteroscedasticity of the distribution of the densities detected through the two methods were tested across sampling units for each category (FMML, mega-fauna and seabirds) using Shapiro-Wilk and Levene tests, respectively. The densities of the three categories did not follow a normal distribution ($p < 0.0001$, Shapiro-Wilk test). The density variances were homogeneous for the FMML ($p = 0.1$, Levene test) and mega-fauna ($p = 0.5$, Levene test) but not for the seabirds ($p = 0.0001$, Levene test). Thus, the densities of the FMML, mega-fauna and seabirds observed by the two methods were compared across the sampling units using a paired-sample Wilcoxon signed-rank test. The densities of the different categories of FMML detected using the two methods were also compared through a non-parametric paired-sample Wilcoxon's signed-rank test. Finally, Spearman's correlation test was used to assess the correlation between FMML density and the distance from the coast and to test whether the two methods could detect such correlations similarly. A $p < 0.05$ significance level was used for all the statistical analyses. The calculations were carried out within the R programming environment (R Core Team, 2014).

3. Results

The environmental parameters and densities of the FMML, mega-fauna and seabirds were variable across the transects, as summarized in Table 2. According to the MEDSEALITTER protocol for FMML aerial monitoring, surveys should be performed with a Beaufort state less than or equal to 3 (MEDSEALITTER consortium, 2019). This condition was satisfied in most transects except for transects 10 and 11, and the sun glare intensity was generally low except for transects 10, 11 and 12. Although the transects established with a Beaufort force >3 and strong sun glare should not be used to determine the FMML distribution and abundance, we included these results in the comparison between the two methods, assuming that they would be affected in a similar way.

3.1. Observer-based survey

A total of 458 targets were detected in the 515 km^2 survey area (Fig. 2 A). The targets mainly consisted of plastic litter items (45.41%, including unidentified items, buoys, boxes, aggregated plastics, bags, buckets and fish boxes), followed by seabirds

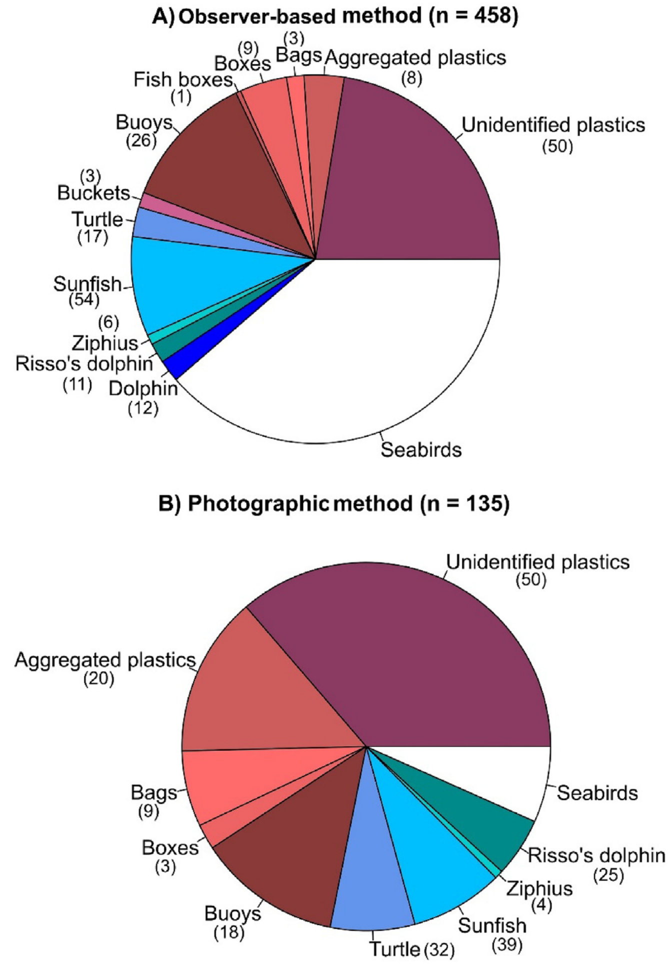

Fig. 2. Marine targets detected by the observer-based method (A) and the photographic method (B) (n = Total targets detected). Purple shades represent FMML categories, blue shades represent mega-fauna species; seabirds are represented in white. The percentage of each category of item/species within its respective category is represented in brackets. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Number of sampling units (n), environmental conditions and densities of marine targets (mean ± SD) split by category and observation method for each transect.

Transect	n	Beaufort force	Sun glare	Clouds (%)	FMML[a] (items/km^2) (mean ± SD)		Mega-fauna (individuals/km^2) (mean ± SD)		Seabirds[a] (individuals/km^2) (mean ± SD)	
					Photographic	Observer-based	Photographic	Observer-based	Photographic	Observer-based
1	7	1–2	1	75	1.56 ± 1.63	0.20 ± 0.18	0.68 ± 1.06	0.37 ± 0.45	0.00 ± 0.00	0.25 ± 0.31
2	9	1–2.5	1	75	1.52 ± 1.44	0.43 ± 0.46	0.51 ± 0.48	0.33 ± 0.49	0.20 ± 0.40	0.15 ± 0.19
3	6	1–2.5	1	80	0.69 ± 1.15	0.40 ± 0.58	0.15 ± 0.37	0.16 ± 0.23	0.00 ± 0.00	0.13 ± 0.24
4	7	1–2.5	0–1	80	0.45 ± 0.58	0.62 ± 0.47	0.39 ± 0.49	0.25 ± 0.27	0.00 ± 0.00	0.17 ± 0.31
5	8	1–2.5	1	10	0.43 ± 1.21	0.07 ± 0.14	0.11 ± 0.32	0.02 ± 0.07	0.00 ± 0.00	0.76 ± 1.17
6	8	1–2.5	1	10	0.30 ± 0.46	0.02 ± 0.07	0.81 ± 2.11	0.39 ± 0.51	0.00 ± 0.00	0.28 ± 0.58
7	7	1–2.5	1	10	0.26 ± 0.45	0.00 ± 0.00	0.26 ± 0.69	0.32 ± 0.53	0.00 ± 0.00	0.09 ± 0.17
8	9	1–3	1	10–50	0.30 ± 0.65	0.19 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.61	0.47 ± 0.41
9	10	2–3	1	50	0.18 ± 0.58	0.00 ± 0.00	0.18 ± 0.39	0.00 ± 0.00	0.18 ± 0.39	1.57 ± 2.12
10	9	2.5–4	1–3	70	0.20 ± 0.61	0.04 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.40	0.65 ± 0.60
11	4	2–5	3	70	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.46	0.47 ± 0.30
12	13	1–2	1–3	10–80	3.22 ± 3.41	1.01 ± 1.52	0.00 ± 0.00	0.02 ± 0.05	–	–
Total					0.90 ± 1.77	0.40 ± 0.84	0.26 ± 0.78	0.15 ± 0.33	0.09 ± 0.31	0.40 ± 0.70

[a] Significant difference between the two methods ($p < 0.05$, paired-sample Wilcoxon's signed rank test).

(38.65%), and mega-fauna (15.94%, represented by sunfish (*Mola mola*), sea turtles (*Caretta caretta*), striped and bottlenose dolphins (*Stenella coeruleoalba* and *Tursiops truncatus*, respectively), Risso's dolphins (*Grampus griseus)*, and Cuvier's beaked whales (*Ziphius cavirostris*)).

3.2. Photographic survey

The images spanned 5184 × 3456 pixels each, and their footprint and GSD were 128.2 m × 86.46 m and 2.5 cm/pixels, respectively. A total of 135 targets were detected in the 10119 images acquired (Figs. 2 B), 71.9% of which were plastic litter items (most of which were unidentified items and aggregated patches and buoys followed by bags and boxes), 20.7% of which were mega-fauna (including sunfish, sea turtles, Risso's dolphins and Cuvier's beaked whale), and 7.4% of which were seabirds. Examples of the vertical images of FMML, mega-fauna and seabirds are shown in Fig. S1.

3.3. Method comparison

The FMML, mega-fauna and seabird densities were compared via a paired-sample Wilcoxon's signed-rank test. The median of the differences between the photographic and observer-based methods across the sampling units was not significantly different from zero for the mega-fauna ($p = 0.75$; Fig. 3 C & D). However, a statistically significant difference was observed for the FMML, which had a higher density when using the photographic method ($p = 0.01$; Fig. 3 A & B), and seabirds, which were better detected by the observer-based method ($p = 0.0001$; Fig. 3 E & F) (Table 2).

The densities of unidentified plastics, aggregated patches and bags detected through the photographic method were significantly higher than those detected through the observer-based method ($p = 0.01$, $p = 0.02$, and $p = 0.04$, respectively, paired-sample Wilcoxon's signed-rank test) (Table 3). However, the densities of buoys, boxes, buckets and fishing boxes detected through the two methods did not statistically differ ($p = 0.36$, $p = 0.13$, $p = 0.06$, and $p = 0.37$, respectively, paired-sample Wilcoxon's signed-rank test) (Table 3).

The FMML density detected through the observer-based method was inversely correlated with the distance from the coast ($\rho = -0.36$, $p = 0.0003$, Spearman's correlation test), but the same correlation was not statistically significant for the FMML density obtained through the photographic method ($\rho = -0.19$, $p = 0.056$, Spearman's correlation test).

4. Discussion

The comparison between the observations obtained through the photographic and the observer-based surveys produced three main results: 1) the photographic method is more effective than the observer-based method for detecting FMML on the sea surface; 2) both methods are equally effective for detecting cetaceans, marine turtles and sunfish; and 3) the observer-based method is more effective than the photographic method for detecting seabirds.

4.1. Floating litter

Aerial monitoring of FMML can be significantly affected by factors such as time of day, sun glare, cloud covering, sea state and wind speed, which may have significant effects on the possibility of detecting floating targets (Colefax et al., 2017). Automatic detection of FMML is also made difficult by its irregular shape and the effect of changing weather conditions on the images (Maire et al., 2013), even though some researchers have recently presented new algorithms that may solve these issues (*e.g.*, Goddijn-Murphy et al., 2018; Kylili et al., 2019).

The majority of our surveys happened with a positive sea state and sun glare conditions, allowing the detection and identification of several categories of FMML through both methods. However, the litter densities detected through aerial photography across the sampling units were on average 2.25 times higher than those detected visually, highlighting a better efficiency of the photographic method.

The observer-based method allowed the identification of more FMML categories than the photographic method, but overall, the densities of unidentified and aggregated items detected by the photographic method were higher. This result may be interpreted as a consequence of the fact that, depending on the conditions in which the photos are taken, floating targets may be better identified by the human eye in real-time than from photographic images. However, as the photographic method allows checking the images several times by multiple photo-interpreters, a higher number of items was detected overall compared to that of the observer-based method, including patches and aggregated items and items that could not be identified at the category level. Instead, the densities of buoys and boxes, which have a positive buoyancy and are more easily detected and identified, were the same for the two methods.

The results obtained from the observer-based survey indicated relatively high FMML densities in sampling sites closer to the coast, consistently with studies highlighting higher FMML densities near the coast than those in the oceanic waters (Ryan, 2014). Indeed, with the exception of the areas located within or near the five ocean gyres (*e.g.,* Lebreton et al., 2018), the highest concentrations of litter are often found in proximity to densely populated urban centres, touristic areas and shipping routes (Suaria and Aliani, 2014). However, this correlation was weaker with the results obtained from the photographic method, probably as a consequence of the smaller area surveyed.

Overall, our results further support the importance of airborne sensors for monitoring the sea surface and detecting floating litter, as already stressed by various authors (Mace, 2012; Pichel et al., 2012; Veenstra and Churnside, 2012). Even if aerial photography is already being used for this purpose at a large scale, including for the monitoring of the "Great Pacific Garbage Patch" (Garaba et al., 2018; Gibbs et al., 2019), the abundances and densities of FMML obtained with photographic methods cannot be included in the databases obtained from observer-based surveys without a previous validation of the methods. Thus, the results of the present study are highly relevant to the comparison of the results obtained from photographic surveys with those obtained from conventional observer-based monitoring of floating litter.

In addition, airborne platforms may be a promising source of evidence-based information for the calibration and validation of future satellite missions aimed at detecting, tracking, identifying, and quantifying ocean plastics (Mace, 2012): photographic surveys for monitoring FMML can be considered a technological intermediary between the satellite- and observer-based methods (Garaba et al., 2018).

4.2. Mega-fauna

Our results show that the observer-based and photographic methods are equally effective for detecting cetaceans, marine turtles and sunfish, providing further validation of photographic surveys as a viable alternative to traditional observer-based surveys for monitoring marine mega-fauna on the sea surface. This result is consistent with similar studies, showing that relevant marine mammal species can be detected and classified to the species level in photographic images (Gibbs et al., 2019; Thaxter & Burton, 2009).

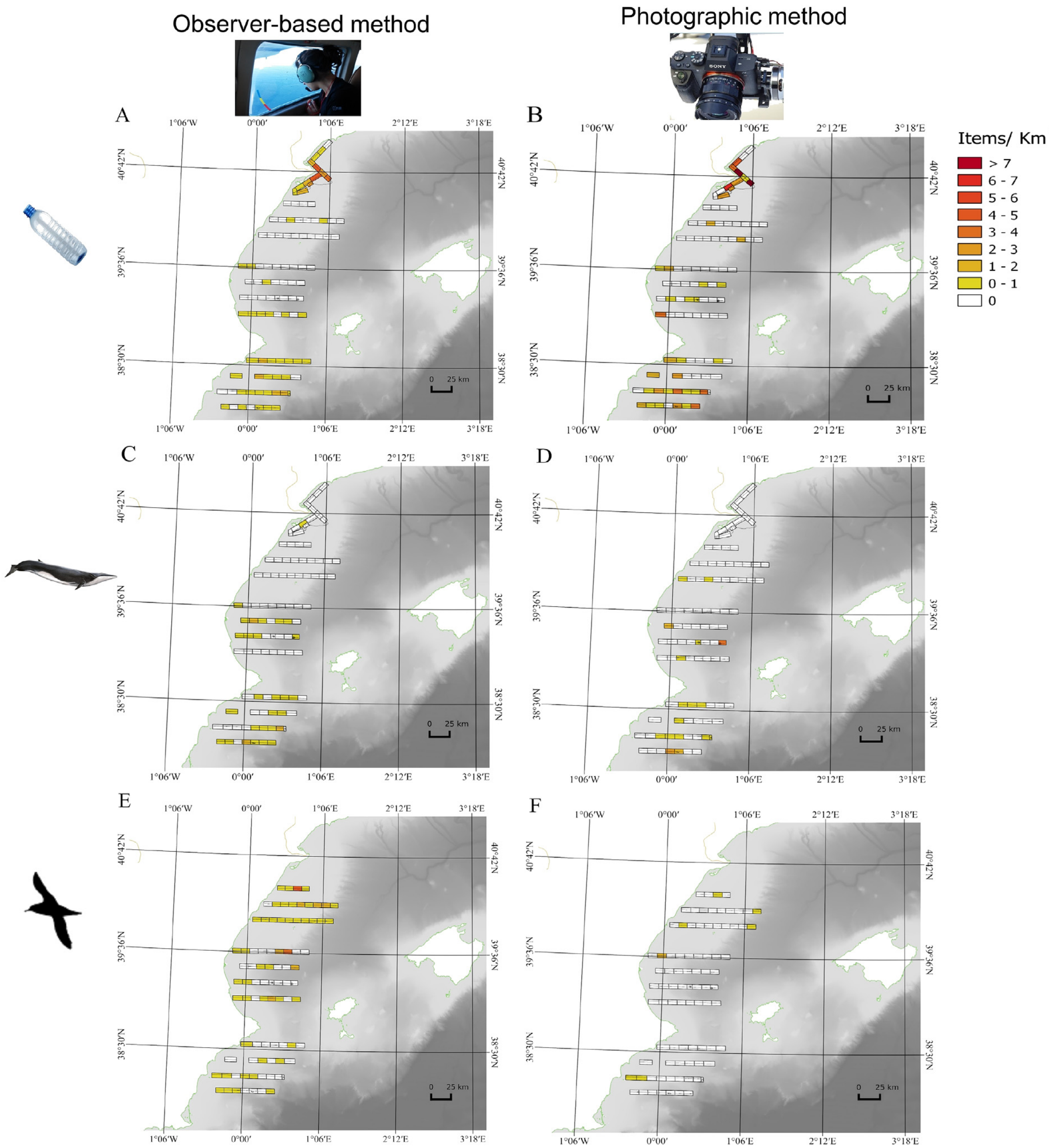

Fig. 3. Density of floating litter (A, B), mega-fauna (C, D) and seabirds (E, F) detected through the observer-based (A, C, E) and photographic (B, D, F) surveys. Sampling units are depicted in each transect.

Taylor et al. (2014) also found that the mean densities of blue shark, loggerhead turtle and ocean sunfish estimated from photographic methods were significantly higher than those estimated from observer-based methods. Such a difference was not highlighted in our study, probably due to the low number of marine mega-fauna observations. As the overall surface of the area that was monitored visually was larger than the area monitored photographically, two dolphin species could be detected only through the observer-based method. It is likely that a larger sample size would reveal significant differences between the ability of two methods to detect the densities of cetaceans, sea turtles and large fish.

Although our results show that the two methods produce comparable results, the advantages of the photographic method also include logistic and economic factors. Observer-based aerial surveys generally require the participation of a number of volunteers or dedicated and trained observers and can sometimes be performed under unsafe conditions (Buckland et al., 2012), and the observations produced cannot be validated afterwards to assess the reliability of the counts and the species identity. An increasing number of national and international regulations (*e.g.*, the Marine Strategy Framework Directive, MSFD 2008/56/EC) require concurrent monitoring of marine mega-fauna and its stressors, including marine litter. These monitoring actions would involve a large number of observers and a massive amount of working hours if performed through observer-based surveys. Photographic surveys, instead, guarantee concurrent monitoring for the presence of

Table 3
Categories of plastic items (total number and density, expressed as items/km^2 ± SD) detected during the photographic and observer-based surveys. In brackets, the number of sampling units considered.

Plastic item category		Photographic survey	Observer-based survey
Unidentified plastics	Total	49	103
	Density (Unidentified plastics/km^2 ± SD; n = 97)	0.45 ± 0.97[a]	0.20 ± 0.43[a]
Aggregated patches	Total	19	16
	Density (Aggregated patches/km^2 ± SD; n = 97)	0.18 ± 0.71[a]	0.029 ± 0.13[a]
Buoys	Total	17	55
	Density (Buoys/km^2 ± SD; n = 97)	0.15 ± 0.75	0.11 ± 0.54
Bags	Total	9	7
	Density (Bags/km^2 ± SD; n = 97)	0.08 ± 0.30[a]	0.01 ± 0.54[a]
Boxes	Total	3	19
	Density (Boxes/km^2 ± SD; n = 97)	0.03 ± 0.18	0.04 ± 0.10
Buckets	Total	0	6
	Density (Buckets/km^2 ± SD; n = 97)	0.00 ± 0.00	0.01 ± 0.54
Fishing boxes	Total	0	2
	Density (Fishing boxes/km^2 ± SD; n = 97)	0.00 ± 0.00	0.01 ± 0.05
Total floating plastic items detected		97	208

[a] Significant difference between the two methods ($p < 0.05$, paired-sample Wilcoxon's signed rank test).

marine fauna and marine litter within the same flights and involve only the pilot and a camera operator. In addition, the analysis of images performed *a posteriori* by trained photo-interpreters allows a better determination of the number of targets and the identification of species and/or items with better precision.

The automatic detection and recognition of targets in the imagery obtained through remote sensing is a key issue of this monitoring technique and may provide further support in locating and identifying marine mega-fauna in the images (Buckland et al., 2012; Bryson & Williams, 2015). Although there are large difficulties in building effective algorithms, some researchers have reached relevant results, developing methods to automatically detect marine animals, birds, rocks and the sea surface (Maussang et al., 2015). Therefore, automated vertical images from aerial platforms open a new horizon of monitoring, and improving technology ensures ever-increasing reliability and quality assurance.

4.3. Seabirds

According to our results, the observer-based method is more effective than the photographic method for detecting seabirds. The apparent contradiction between this result and those obtained for the FMML and mega-fauna may be explained by three main factors. 1) While cetaceans, fish and sea turtles can be observed only on the sea surface and in the few centimetres below it, seabirds can be observed not only floating at sea but also flying in the three-dimensional aerial space between the aircraft and the sea surface. Being equal the ground surface, the observer-based monitoring methods cover larger volumes of space than the photographic methods. 2) The photographic surveys did not generate sufficient seabird observations to perform a proper density comparison between the methods. 3) Flying birds remain within the field of view of the camera for short periods of time, whereas observers are able to follow moving targets for longer periods of time.

Hence, a possible solution to overcome at least one of these biases may be to cover larger areas to obtain comparable observations. Other studies comparing the two methods indicated that seabird surveys conducted using aerial photography can be more accurate than those conducted with observers (Chabot and Francis, 2016). For instance, Žydelis et al. (2019) recorded more bird sightings, identified more species and detected higher densities of nearly all species through digital video surveys than with concurrent observer-based surveys. In addition, the results from Kulemeyer et al. (2011) suggested that the frequencies of three sea duck species were underestimated by an observer-based method, being lower than those determined through an aerial photographic method. According to these authors, aerial photography may prove to be the tool of choice to identify seabird species and to precisely count individuals in large groups, whereas the human eye may allow only a rough estimate (Žydelis et al., 2019). However, in the present study, birds were not identified at the species level with either of the two methods, and no large groups of seabirds were encountered. Thus, our results suggest that in areas of scarce bird density, three-dimensional visual observations may record more individuals than bi-dimensional aerial photography.

To overcome the limitations of aerial photography described above, UAVs are frequently used for seabird monitoring (*e.g.*, Brisson-Curadeau et al., 2017; Weimerskirch et al., 2018). Drones may provide an effective alternative to aircraft for the following reasons: 1) they can fly at lower altitudes, leading to an increase in image resolution; 2) they can be programmed to take several pictures per second, which allows a continuous overlap between photographs; 3) image processing programs (*e.g.*, Agisoft PhotoScan) can produce georeferenced orthomosaics from the overlapped photographs, providing a single high resolution image of the surveyed areas; 4) the weight, cost and environmental footprint of drones are reduced compared to those of aircraft; and 5) the risks for the pilot and researchers are null (Bryson & Williams, 2015). On the other hand, the average endurance of UAVs is generally limited compared to that of manned aerial vehicles, which are able to cover larger areas.

4.4. Time effort

To properly compare the observer-based and photographic methods, it is necessary to calculate the overall time effort needed for data collection and processing within the two methods. The observer-based method needs a standard team of two to three observers (if one is dedicated exclusively to marine litter) and a person in charge of recording data and organizing the database afterwards, leading, for an 8-h survey, to an overall time requirement ranging between 26 and 34 h (8 h per person per survey plus 2 h for data management).

On the other hand, the photographic method needs a camera operator and one or two photo-interpreters, leading, for an 8-h survey in which approximately 2500 images are taken, to an overall effort of approximately 24 h (8 h for the camera operator plus 14 h for photo interpretation and 2 h for the inspection of doubtful targets).

Although the time required for the two methods is of the same order of magnitude, the effort is slightly reduced for photographic surveys, which is another reason to consider the photographic method a viable alternative to observer-based methods. Moreover, the development of new, efficient algorithms to automatically detect targets will further reduce the effort dedicated to manually inspecting the images (Bryson & Williams, 2015).

5. Conclusions

The results of this paper provide a first validation of the photographic method for FMML monitoring, enabling the comparison of data obtained through this method with those obtained from observer-based methods and thus the determination of temporal trends in marine mega-fauna and FMML density and distribution. The increasing application of photographic methods for monitoring the marine surface is supported by a number of factors, including the constant improvement of technology, ensuring the reliability and quality of data, and the development of automated algorithms that will allow the analysis of thousands of images per hour.

Our results indicate that for FMML and mega-fauna (with the exception of seabird) monitoring, the photographic method is equally as efficient as or more efficient than the observer-based method. The use of manned aerial vehicles is recommended for the purpose of monitoring large spatial scales, while the use of UAVs is recommended for relatively small-scale monitoring and/or when more accurate data are needed. However, further research is needed to select the best devices for identifying floating litter, to cope with the issue of sun glare reflection, and to improve the currently available algorithms.

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Appendix A. Supplementary data

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1.3. Automatic detection and quantification of floating marine macro-litter in aerial images: Introducing a novel deep learning approach connected to a web application in R

Autores: Odei Garcia-Garin[1], Toni Monleón-Getino[2,3,4], Pere López-Brosa[2,3], Asunción Borrell[1], Alex Aguilar[1], Ricardo Borja-Robalino[2,3], Luis Cardona[1], Morgana Vighi[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain
[2]Department of Genetics, Microbiology and Statistics, Universitat de Barcelona, Barcelona, Spain
[3]BIOST3, Spain
[4]GRBIO (Research Group in Biostatistics and Bioinformatics), Barcelona, Spain

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Abstract:
The threats posed by floating marine macro-litter (FMML) of anthropogenic origin to the marine fauna, and marine ecosystems in general, are universally recognized. Dedicated monitoring programmes and mitigation measures are in place to address this issue worldwide, with the increasing support of new technologies and the automation of analytical processes. In the current study, we developed algorithms capable of detecting and quantifying FMML in aerial images, and a web-oriented application that allows users to identify FMML within images of the sea surface. The proposed algorithm is based on a deep learning approach that uses convolutional neural networks (CNNs) capable of learning from unstructured or unlabelled data. The CNN-based deep learning model was trained and tested using 3723 aerial images (50% containing FMML, 50% without FMML) taken by drones and aircraft over the waters of the NW Mediterranean Sea. The accuracies of image classification (performed using all the images for training and testing the model) and cross-validation (performed using 90% of images for training and 10% for testing) were 0.85 and 0.81, respectively. The Shiny package of R was then used to develop a user-friendly application to identify and quantify FMML within the aerial images. The implementation of this, and similar algorithms, allows streamlining substantially the detection and quantification of FMML, providing support to the monitoring and assessment of this environmental threat. However, the automated monitoring of FMML in the open sea still represents a technological challenge, and further research is needed to improve the accuracy of current algorithms.

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Automatic detection and quantification of floating marine macro-litter in aerial images: Introducing a novel deep learning approach connected to a web application in R☆

Odei Garcia-Garin [a, *], Toni Monleón-Getino [b, c, d], Pere López-Brosa [b, c], Asunción Borrell [a], Alex Aguilar [a], Ricardo Borja-Robalino [b, c], Luis Cardona [a], Morgana Vighi [a]

[a] Institute of Biodiversity Research (IRBio) and Department of Evolutionary Biology, Ecology and Environmental Sciences, Universitat de Barcelona, Barcelona, Spain
[b] Department of Genetics, Microbiology and Statistics, Universitat de Barcelona, Barcelona, Spain
[c] BIOST[3], Spain
[d] GRBIO (Research Group in Biostatistics and Bioinformatics), Barcelona, Spain

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A B S T R A C T

The threats posed by floating marine macro-litter (FMML) of anthropogenic origin to the marine fauna, and marine ecosystems in general, are universally recognized. Dedicated monitoring programmes and mitigation measures are in place to address this issue worldwide, with the increasing support of new technologies and the automation of analytical processes. In the current study, we developed algorithms capable of detecting and quantifying FMML in aerial images, and a web-oriented application that allows users to identify FMML within images of the sea surface. The proposed algorithm is based on a deep learning approach that uses convolutional neural networks (CNNs) capable of learning from unstructured or unlabelled data. The CNN-based deep learning model was trained and tested using 3723 aerial images (50% containing FMML, 50% without FMML) taken by drones and aircraft over the waters of the NW Mediterranean Sea. The accuracies of image classification (performed using all the images for training and testing the model) and cross-validation (performed using 90% of images for training and 10% for testing) were 0.85 and 0.81, respectively. The Shiny package of R was then used to develop a user-friendly application to identify and quantify FMML within the aerial images. The implementation of this, and similar algorithms, allows streamlining substantially the detection and quantification of FMML, providing support to the monitoring and assessment of this environmental threat. However, the automated monitoring of FMML in the open sea still represents a technological challenge, and further research is needed to improve the accuracy of current algorithms.

1. Introduction

Marine litter, defined as *any persistent, manufactured or processed solid material discarded, disposed of, abandoned, or lost in the marine and coastal environment* (UNEP, 2005), is ubiquitous in all marine compartments worldwide (*e.g.*, Arcangeli et al., 2018; Cózar et al., 2014; Suaria et al., 2020). It poses a potential threat to the marine fauna, including invertebrates (*e.g.*, Digka et al., 2018), fish (*e.g.*, Garcia-Garin et al., 2019; 2020d), marine mammals (*e.g.*, De Stephanis et al., 2013), and turtles (*e.g.*, Schuyler et al., 2014). Floating marine macro-litter (FMML, *i.e.*, objects > 2.5 cm; Galgani et al., 2013; GESAMP, 2019) of anthropogenic origin is particularly harmful, because of its potential to entangle all sort of marine organisms (*e.g.*, fishes, turtles, marine mammals; Deudero & Alomar, 2015), and of being ingested by marine fauna, especially large filter-feeding species (Garcia-Garin et al., 2020c). Monitoring its density and distribution patterns through standardized methodologies (Van Sebille et al., 2020) is highly needed to assess the extent of this environmental threat (GESAMP, 2015; UNEP 2016).

FMML presence and distribution have been traditionally assessed through manta trawl nets (*e.g.*, Lebreton et al., 2018), indicator species (*e.g.*, Domènech et al., 2019), and observer-based

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* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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methods applied from vessels (Arcangeli et al., 2018; Suaria and Aliani, 2014) or manned aircraft (*e.g.*, Garcia-Garin et al., 2020a; Pichel et al., 2012), which are often time-demanding and expensive techniques. Although traditional observer-based methods present many advantages (*e.g.*, precise identification of targets, absence of constraints related to the duration of the camera battery charge or the storage space), alternative remote sensing methods offer distinct advantages, such as more objective and reproducible results, and the possibility to re-analyse the recorded images for other investigations (Garcia-Garin et al., 2020b; Veenstra and Churnside, 2012). Thus, either passive (*e.g.*, RGB video, digital camera, multispectral, hyperspectral) or active (*e.g.*, lidar, radar) sensors coupled to aerial vehicles (*e.g.*, aircraft, drones, satellites) can be excellent tools to quantify and monitor the distribution of FMML (Garcia-Garin et al., 2020b; Kikaki et al., 2020; Martínez-Vicente et al., 2019; Maximenko et al., 2019; Veenstra and Churnside, 2012; Topouzelis et al., 2019). Nevertheless, these techniques can also be highly time-consuming if the analysis of the images is done manually by one or more trained scientists, (*e.g.*, Garcia-Garin et al., 2020a). The development of algorithms to automatically detect FMML in aerial images, and thus streamline the analytical process, is critical for the successful implementation of these techniques.

In the last decade, machine learning models have shown good results in the analysis of environmental processes (Quetglas et al., 2011). In particular, deep learning models using Convolutional Neural Networks (CNNs) have been widely applied due to their ability to recognize features and patterns contained in large datasets of images or videos (Guirado et al., 2019; Velandia et al., 2017). So far, few algorithms have been developed to detect and identify FMML in digital images (Kylili et al., 2019). To the best of our knowledge, none of them were trained or tested to recognize floating litter items using aerial RGB images. Some authors (*e.g.*, Garaba and Dierssen 2018; Garaba et al., 2018; Goddijn-Murphy and Dufaur, 2018; Goddijn-Murphy et al., 2018; Kikaki et al., 2020; Topouzelis et al., 2019) used spectral information to develop models that could automatically detect litter items and could be applied to aerial imagery, and others (*e.g.*, Kylili et al., 2019) successfully applied CNN models to automatically detect FMML in images taken few meters above the water surface. Remote sensing of FMML is in its infancy (Garaba et al., 2018; Maximenko et al., 2019), and despite recent improvements and encouraging results, algorithms able to automatically detect FMML in aerial RGB images are still lacking.

The aim of the present study was to develop an R (R Core Team, 2020) library based on a deep learning approach, to automatically detect and quantify FMML in aerial images of the sea surface taken from drones and aircraft. After validating the accuracy of the package, we also propose the implementation of such approach through a web-oriented application based on the Shiny package. The development of user-friendly applications for monitoring the presence of floating marine litter would facilitate the implementation of routine monitoring programmes of this threat, in compliance with current regional and national environmental regulations.

2. Materials and methods

2.1. Survey area

Aerial images were obtained during photographic surveys performed by drones and manned aircraft between 2017 and 2019 over the marine area located between *Delta de l'Ebre* and *Cap de Creus* (NW Mediterranean, Fig. 1). Surveys with drones were performed on May 16th and June 3rd 2017 at Blanes, June 6th 2018 at *Cap de Creus* and February 4th 2019 *at Delta de l'Ebre*. Surveys with aircraft were performed on January 24th and March 14th 2018 at *Delta de l'Ebre*. To minimize the effect of wind and sun glint on the detection

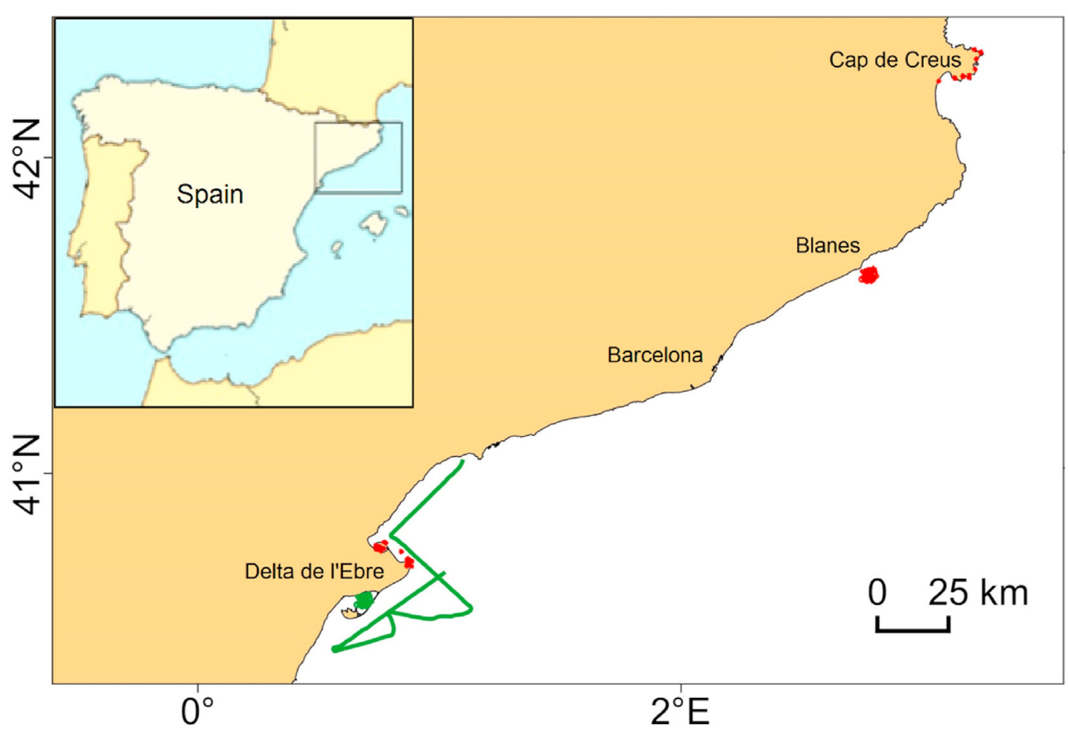

Fig. 1. Map of the study area indicating the GPS tracks and locations of the drone (red) and aircraft (green) surveys performed over the areas of *Cap de Creus*, Blanes and *Delta de l'Ebre*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

of FMML, all surveys were conducted with low wind force (*i.e.*, Beaufort sea state < 3) and avoiding the hours of the day when the sun was higher on the horizon.

2.2. Photographic surveys

2.2.1. Drone surveys

Four different types of drones were used: (1) a fixed-wing HP1 equipped with an RGB camera Sony ILCE-6000 (6000 × 4000 pixels), and (2) a multi-rotor *Topografia* equipped with an RGB camera Sony Alpha 7 R (7952 × 5304 pixels) off Blanes, (3) a Phantom 3 Advanced equipped with an RGB camera FC300S (4000 × 3000 pixels) at *Cap de Creus*, and (4) a DJI Mavic Pro equipped with an RGB camera FC220 (4000 × 3000 pixels) at *Delta de l'Ebre*. All images were taken with the cameras placed in the nadir position at altitudes ranging from 20 to 120 m, and with a ground sampling distance ranging between 0.6 and 3.6 cm pixel^{-1}. A total of 3900 images were recorded, of which the 121 taken by (1) and 200 by (2), were shot over positive controls (*i.e.*, a series of FMML of known size and type) deployed from a boat (Fig. 2). The remaining 2589 images taken by (3) and 990 by (4) were recorded over natural sea conditions.

2.2.2. Aircraft surveys

Aircraft surveys were performed with a high-wing aircraft (Partenavia P- 68) flying at a constant groundspeed of 90 knots (166 km h^{-1}) and an altitude ranging from 230 to 300 m, equipped with a Canon EOS REBEL SL1 (5184 × 3456 pixels) camera connected to the aircraft GPS signal. The camera, placed under the aircraft and pointed at 90° to the ground, was controlled from a tablet through the Waldo Flight Control System software. Ground sampling distance ranged between 2.5 and 3.3 cm pixel^{-1}. A total of 3000 images were taken, 25 of which were obtained over the same positive controls used for the drone surveys (Fig. 3A).

2.3. Image pre-processing

Images were inspected by a trained scientist to detect the presence of FMML, and a subset of 796 images was labelled according to the following categories: (1) containing FMML (398 images), and (2) not containing FMML (398 images). The trained scientist had a proven experience in detecting floating litter in aerial imagery as he had previously reviewed thousands of images for the purpose and he was also involved in the field observations. However, all doubtful items were checked by a second experienced researcher. As thousands of images per category are usually needed to train properly a deep learning model (Sun et al., 2017), the available images for each category were not enough. Thus, the number of images was increased through data augmentation (*i.e.*, shifting, zooming, rotation, etc. of the available images) as in Kylili et al. (2019), to obtain a larger dataset of 1860 images containing FMML and 1863 images without FMML. Examples of aerial images taken by drones and aircraft over positive controls, FMML and sea water, are shown in Fig. 3.

2.4. Deep learning algorithm

Consistently with Kylili et al. (2019), an algorithm to automatically detect FMML in aerial images was developed by applying a deep learning approach based on a CNN architecture. While other

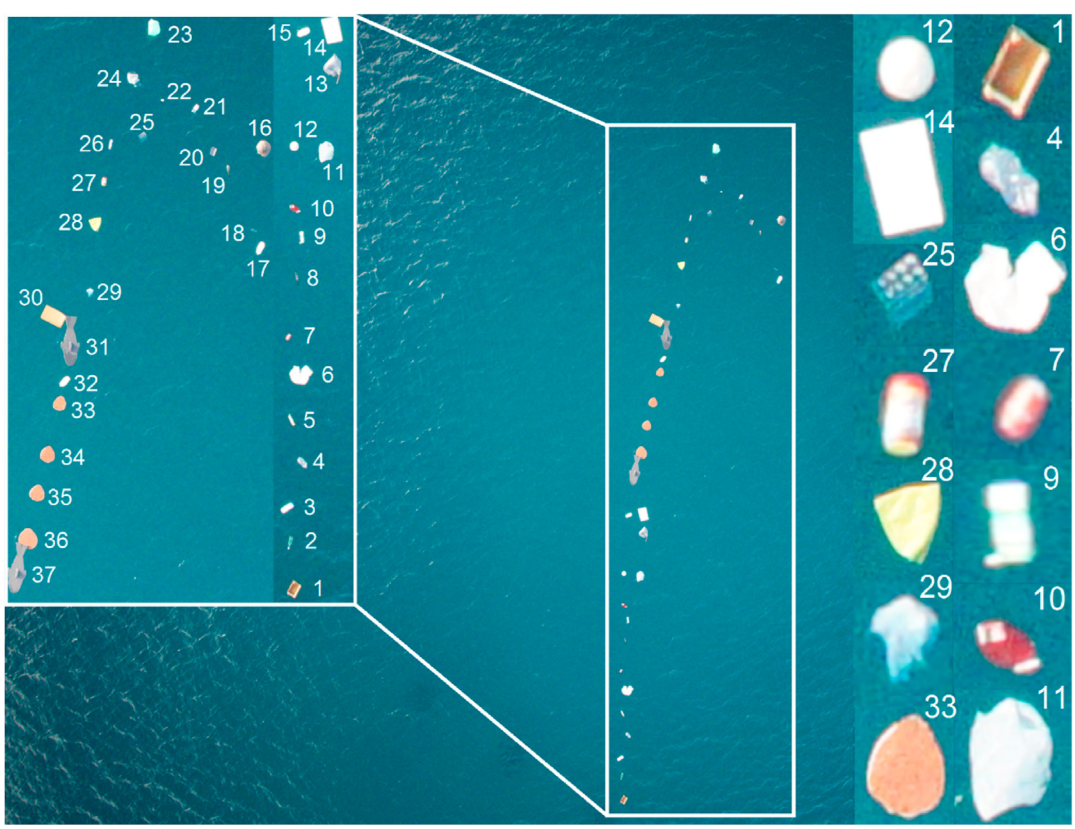

Fig. 2. Example of an aerial image taken by the drone over a series of known items with neutral or positive buoyancy attached to a line deployed from a boat (*i.e.*, our positive control). 1 = crate; 2–4, 8, 19 = bottles; 5, 7, 22, 26 = cans; 6 = sack; 9, 21 = tetra-brik packages; 10 = net; 11,13, 23, 24 = bags; 12 = ball; 14, 30 = boards; 15, 17, 32 = drum; 16 = turtle carapax; 18 = six pack rings; 20, 25 = trays; 27 = jar; 28 = balloon; 29 = towels; 31, 33–37 = polystyrene.

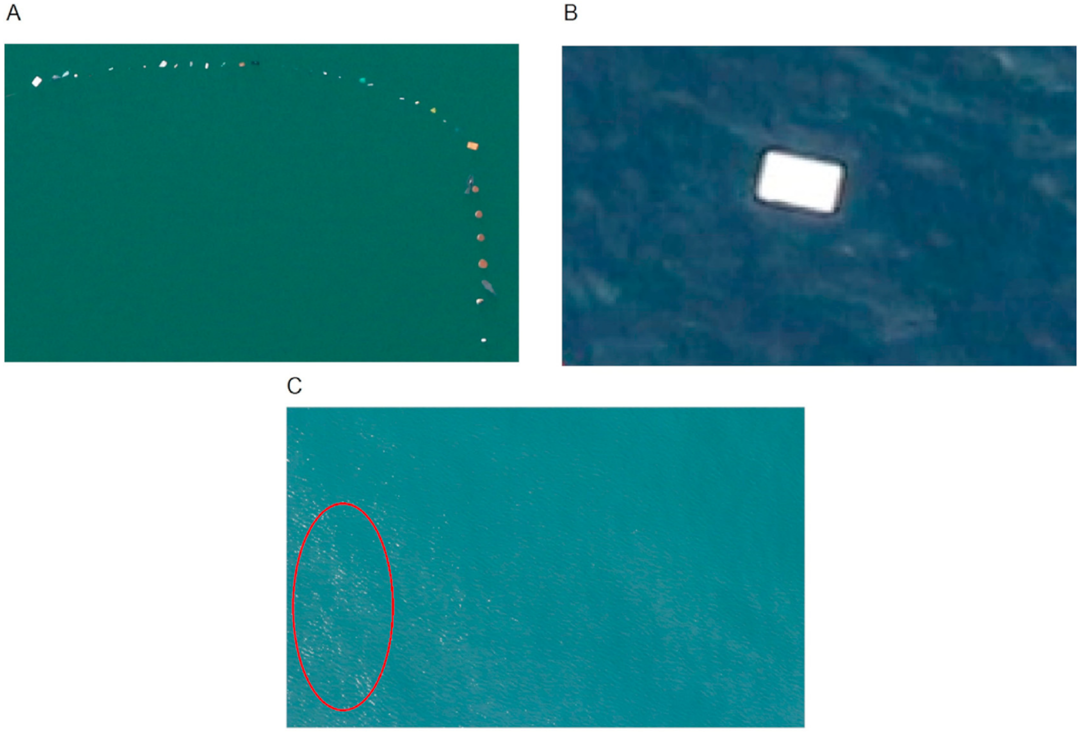

Fig. 3. Examples of aerial images ((A) positive controls as in Fig. 2, (B) board and (C) sea water with an area affected by sun glint circled in red) taken from aircraft and drones. Images were cropped to improve the visibility of items. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

machine learning methods need a set of features to feed the classifier, deep learning based on CNNs trains and recognizes the spatial patterns of the targets using a series of features that are inside its native structure (Gonçalves et al., 2020b; Guirado et al., 2019).

A total of 34 different models (Table S2) were developed in R (R Core Team, 2020) with the following CNN architectures:

Architecture 1: 1 Convolutional layer (Hyperparameters) + 1 Pooling layer (size = 1 × 1) + 1 Convolutional layer (Hyperparameters) + 1 Pooling layer (size = 1 × 1) + 1 Fully connected (500 neurons, Tanh) + 1 Fully connected (20 neurons) + Softmax.

Architecture 2: 2 Convolutional layers (Hyperparameters) + 1 Pooling layer (size = 2 × 2) + 2 Convolutional layers (Hyperparameters) + 1 Pooling layer (size = 2 × 2) + 1 Fully connected (3200 neurons, ReLU) + 1 Fully connected (1000 neurons) + Softmax.

After testing the different models (Table S2), the second CNN architecture, showed in Fig. 4, was selected as the best option. The optimization of the value of the hyperparameters was decisive to obtain the maximum accuracy in the training and testing sets without falling into overfitting. The optimal parameters were: Kernel = 3 × 3, learning rate = 0.0001, batch size = 100, momentum = 0.9, optimizer = Adam, epochs = 400.

Such architecture was built through the use of three types of layers: (1) the convolutional layer, which extracts features from the input images at different levels of hierarchy, (2) the pooling layer, which is a reduction operation used to increase the abstraction level of the extracted features, and (3) fully connected and hidden layers, which are used as classifiers at the end of the pipeline (Fig. 4). Convolutional layers were composed of the convolution of small groups of pixels (3 × 3) extracted from the input image by a kernel matrix with the addition of a bias; these parameters were previously established during the network learning process. The ReLU activation function, which complies with the basic property of introducing non-linearity in the system (Shridhar et al., 2019; Velandia et al., 2017), was applied to facilitate the optimization process for binary classifications. A pooling layer was also applied to each convolutional layer to perform a subsampling process with the most relevant features. A process of vectorization and concatenation of data (flattened) was carried out, allowing the application of two completely connected layers that made the convolutional layers determine the learning process of the most relevant characteristics.

The softmax regression, which is often used in neural networks to map the non-normalized output to a probability distribution of a defined number of predicted outputs, was used to obtain the prediction of 2 categories (*i.e.*, FMML, no FMML). The softmax function (Bishop, 2006) took as input a vector of K real numbers from the hidden layers and normalized them into a probability distribution consisting of K probabilities proportional to the exponentials of the input numbers. The learning of the neural network was done through the backpropagation process. Thus, the parameters were estimated and updated until the network reached the optimal solution through the estimation of the weight matrix and the vector of biases within the hyperconus of feasible solutions (Fig. 5) that allowed the convergence of the model with the best performance (Shridhar et al., 2019; Velandia et al., 2017).

A library named AIImagePred (automatic image recognition and prediction based on deep learning) was developed in R (R Core Team, 2020) to classify images in 2 classes, based on the CNN model. AIImagePred was developed based on other well-known libraries in the deep-learning environment, such as Keras (Falbel et al., 2018) and Mxnet (Chen et al., 2018), as a general-purpose package for image recognition and prediction based on CNNs. As AIImagePred includes an algorithm that splits the image into multiple parts, individual or multiple images can be processed, without prior separation or segmentation.

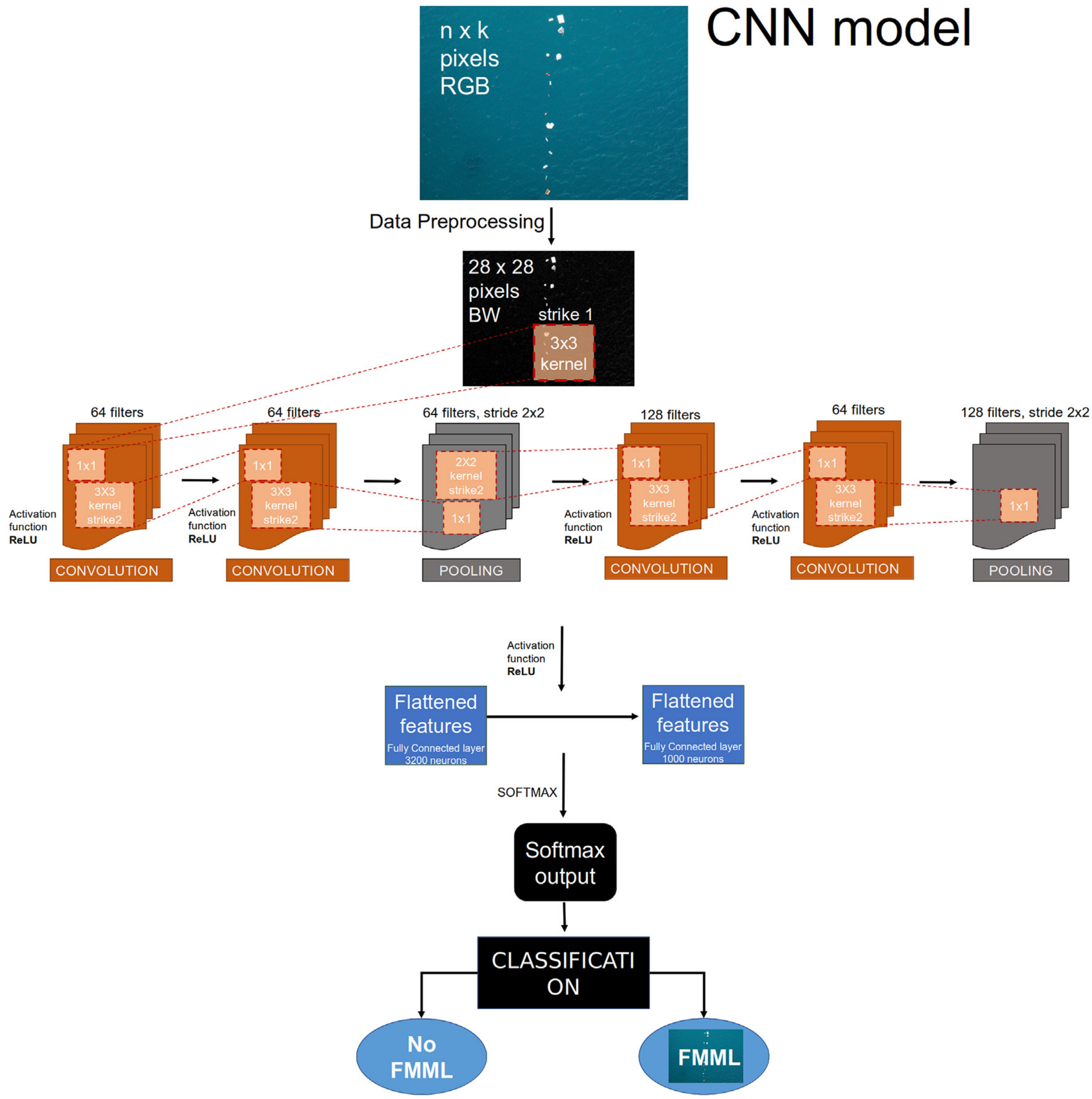

Fig. 4. Convolutional neural network workflow. The processed image was a positive control image of marine litter captured from a drone.

The two main functions of AIImagePred (Table 1) were: (1) image. trainimages.DL.algorithm_multiclass(), which creates and trains the CNN model for the classification of aerial images based on the architecture in Fig. 4; and (2) split. predict.count.multiclass(), which uses the pre-trained CNN model to classify a testing set of images in a series of 2 predefined classes. Thus, once the image is split in multiple cells (we recommend splitting images into at least 25 cells), the algorithm classifies each cell of the image as containing FMML or not.

To guarantee the functionality of the algorithm, aerial images must be taken in nadir position with a ground sampling distance of at least 3.6 cm pixel^{-1}. To further improve the algorithm accuracy, aerial images should be taken with sea state conditions of Beaufort < 3 and avoiding the times of the day when the sun is higher on the horizon to minimize the effect of the wind and the sun glint on the detection of FMML.

The convolutional layering and pooling operations are represented by Equation (1). The resulting matrices of the convolutional layers (C) were flattened. Equation (2) represents the complete model including the densely connected layers (Dumoulin and Visin, 2018; Kuo, 2016):

$$C = P\left[a\left(\sum_{q=1}^{128} a\left(\sum_{q=1}^{128}\left(P\left[a\left(\sum_{q=1}^{64} a\left(\sum_{q=1}^{64} I*K_{1,q}^{1}+b_q^1\right)*K_{1,q}^{2}+b_q^2\right)\right]_{\max(2i,2j)}\right)*K_{1,q}^{3}+b_q^3\right)*K_{1,q}^{4}+b_q^4\right)\right]_{\max(2i,2j)} \quad (1)$$

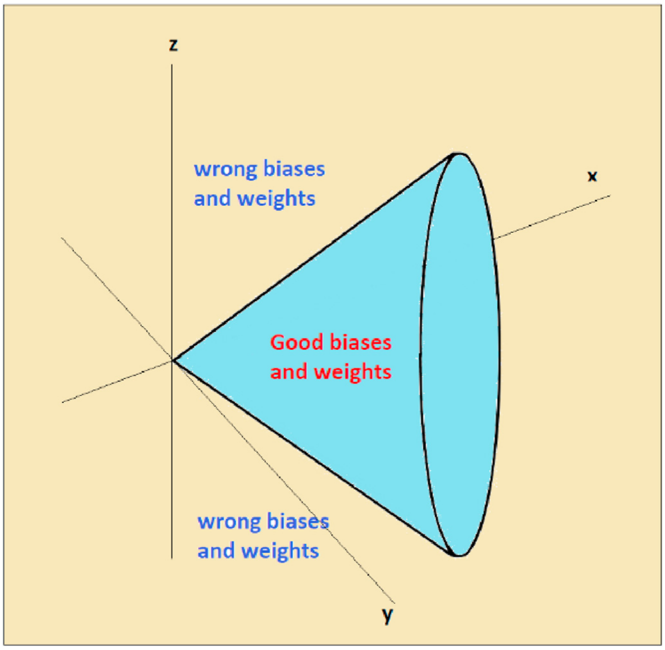

Fig. 5. Three-dimensional region of feasible solutions for weights and biases in the case of binary classification. The axes represent the dimensions in the convolutional space (x = length, y = width, z = depth). Each point within the cone represents the optimal value for each convolutional layer to extract the characteristics of the image in the best way.

$$\hat{y} = softmax\left[\sum_{q=1}^{1000}\left[a\left(\sum_{q=1}^{3200} C_a \cdot K_{1,q}^{5} + b_q^5\right)\right] \cdot k_{1,q}^{6} + b_q^6\right] \tag{2}$$

where:

- $K_{p,q}^{l}$ = Kernel matrix; l = number of the layer; p = origin; q = filter number.
- b_q^l = biases.
- $P_{max(2i,2j)}$ = pooling layer (max-pooling).
- a = activation function “ReLU”.
- $\hat{y}$ = output (FMML − not FMML).
- $(*)$ = convolution; $(\cdot)$ = matrix product.
- C = convolution and pooling layer process.
- C_a = vectorization and concatenation of the output C.

2.5. Accuracy assessment

A total of 3723 images (1860 with FMML, 1863 without FMML) were used to train/test the model during classification, 90% and 10% of which were used to train and test the model, respectively, during cross-validation. The overall accuracy of the model results was assessed during both processes through four parameters: accuracy, precision, recall, and F1-score. Accuracy (Equation (3)) represents the fraction of all the images processed that were correctly classified as containing FMML or not containing FMML, precision (Equation (4)) represents the fraction of images classified as containing FMML that actually belonged to that class, while recall (Equation (5)) represents the fraction of correctly labelled images within each class. Accuracy, precision, and recall values vary between 0 and 1. F1-score (Equation (6)) represents a balance between precision and recall (Fawcett, 2006) and its value increases with the performance of the model (Bekkar et al., 2013). The accuracy assessment was obtained from the training and testing sets over 400 epochs.

$$Accuracy = \frac{TP + TN}{N} \tag{3}$$

$$Precision = \frac{TP}{TP + FP} \tag{4}$$

$$Recall = \frac{TP}{TP + FN} \tag{5}$$

$$F1 = \frac{2{*}TP}{2{*}TP + FP + FN} \tag{6}$$

where:

- TP = True positive: images with FMML well classified
- TN = True negative: images without FMML well classified.
- FP = False positive: images without FMML misclassified.
- FN = False negative: images with FMML misclassified.
- N = Total images analysed.

The repeatability of the method was tested by processing 10 runs of randomly selected image sets (n = 3723, 90% of which were used for training, and 10% for testing).

2.6. Application based on the Shiny package

An interactive web application was built in R, based on a simpler version of the CNN model developed (see supplementary material:

Table 1
Functions contained in the library AIImagePred.

Function	Features	Arguments
image.trainimages.DL.algorithm_ multiclass()	Algorithm that extracts features from the image to train a CNN-based deep learning model using the architecture presented in Fig. 4. The algorithm creates, tests, and optimizes the model to be used for image classification.	Function1(dir.imag.training, dictionary = c(plastic = 0, sin = 1), size_foto = 28, check.accuracy = T, Do.saveRDS = T, Do.save.model = T, percent.CV = 0.9, num.round = 400)
split.predict.count.multiclass()	Algorithm that automatically identifies FMML in aerial images: it reads the trained images, and predicts their class. The algorithm allows the analysis of the whole image or its division in equal parts, after converting it to multiple images.	Function2(train.images.dir, name.files.images, predict.test.images.dir, size_foto = 28, dictionary = c(plastic = 0, sin = 1), use.model = T, nom.model.saved = “prova_model.RData”, num.round = 400, n.div = 5, my.opinion = NULL).

Table S2, test 2 & Fig. S1), using the Shiny package (Chang et al., 2020). The scope of the application was to create a user-friendly interface that could allow the detection of FMML in any aerial image that is uploaded by the user.

2.7. Hardware requirements

Image processing and numerical calculations under pre-built or designed CNN architectures require high-level processors with special features in their RAM and graphic cards (NVIDIA). Currently, the most popular mean of developing Artificial Intelligence is the computer running NVIDIA, closely followed by Raspberry Pi. Due to the high computational cost required to train the network using cross-validation and the high quality of the images analysed, the pre- and post-processing of the network were developed using a HPC Computer Server, 40 cores Xeon SP 4114 2,2 GHz, within the premises of the University of Barcelona (Spain).

Despite the new Raspberry Pi 4 - Model B of 8 gigabytes could allow the classification of images (testing phase), this option was discarded due to its too expensive training in the metric “execution time".

3. Results

3.1. CNN model accuracy

The function image.trainimages.DL.algorithm_multiclass() was used to compute the CNN model, following the CNN architecture presented in Fig. 4. The accuracy of the CNN model was tested during classification and cross-validation. In a first step, all the labelled images were used to test the total accuracy (n = 3723, 1860 images with FMML, 1863 images without FMML). The classification accuracy was 0.85 (TP = 94%, TN = 76%) using all images as both training and testing set, and 0.81 (TP = 84%, TN = 78%) after cross-validation (n = 3723, 90% images used for training, 10% for testing) (Table 2). The maximum accuracies attained by cross-validation during training and testing were 0.90 and 0.85, respectively. Images mis-classified were those that were most affected by sun glint (Fig. 3B). It should be noted that the accuracy obtained during cross-validation was lower than that of classification because the first process uses different images for training and testing, while the same images are used for training and testing during classification.

The repeatability of the method was tested by processing 10 runs of randomly selected image sets (n = 3723, 90% images used for training, 10% for testing) using the AIImagePred function and computing the accuracy for each set. The mean classification accuracy was 0.85 ± 0.03 for the training sets and 0.79 ± 0.03 for the testing sets (Fig. 6).

3.2. Application based on Shiny language

A visual application, namely MARLIT, to detect and quantify FMML in aerial images, oriented to web applications, was developed through the Shiny package within the R programming language. The web application, accessible from a computer device, allowed: (1) uploading aerial images; (2) splitting images into multiple cells, (3) analysing them through the AIImagePred R library; (4) detecting FMML presence in each of the cells; and (5) quantifying its density in relation to the surface covered by the images, which is calculated from the metadata provided in the uploading phase, namely the height and focal distance. They are online for public test and use, and any possible improvements or suggestions from other researchers are warmly welcomed. The CNN model analyses each cell separately to determine if it contains FMML or not. By increasing the number of cells, the accuracy of the FMML density calculated by the application is improved, but the time needed for processing increases. The MARLIT application and the AIImagePred library can be downloaded from https://github.com/amonleong/MARLIT.

Fig. 7 shows an example of the application interface, where an image containing FMML is analysed, cells containing FMML are identified and FMML relative density is quantified.

4. Discussion

In this study, we applied CNN-based deep learning models to detect and quantify FMML in aerial images, we proposed their coupling to the AIImagePred library in R and their implementation on a web-oriented application based on the Shiny package. Results obtained from the application of the optimal CNN model to analyse 3723 aerial images recorded during drone and aircraft surveys showed good accuracies of FMML detection.

Our results further support the use of airborne sensors for inspecting the sea surface and detecting FMML. Studies based on these techniques for FMML monitoring have substantially increased within the last decade. Aerial photography is already being used for this aim at large scale, including for the monitoring of the “Great Pacific Garbage Patch” (Garaba et al., 2018; Lebreton et al., 2018) and of coastal areas of the Western Mediterranean Sea (Garcia-Garin et al., 2020a). However, when photographic methods are used, densities of FMML are not calculated through a common, standardized, and efficient algorithm, since image analyses are still often performed manually. Aerial photography methods should be coupled with efficient automated FMML detection processes to prove their effectiveness and to provide a valid alternative to replace traditional monitoring techniques.

The remote sensing of marine litter is a technological challenge and is currently in constant development (Martínez-Vicente et al.,

Table 2
Accuracy, precision, recall and F1-score of the CNN model here proposed to detect FMML in aerial RGB images and of those currently available for marine litter detection. * Mean values are shown.

	Method	Process	Accuracy	Precision	Recall	F1-score
This study	CNN	Classification	0.85	0.79	0.94	0.86
	CNN	Cross-validation (training)	0.85	0.81	0.91	0.86
	CNN	Cross-validation (testing)	0.81	0.82	0.84	0.83
Martin et al. (2018)	Random forest		–	0.08	0.40	0.13
Fallati et al. (2019)	CNN	Training	0.95	–	–	–
	CNN	Testing	-	0.54	0.44	0.49
Kylili et al. (2019)	CNN	Training	1	–	–	–
	CNN	Testing	0.99	-	-	-
Jakovljevic et al. (2020)*	CNN		–	0.82	0.59	0.66
Gonçalves et al. (2020a)	Random forest		–	0.73	0.74	0.75
Gonçalves et al. (2020b)	CNN		–	0.55	0.65	0.60

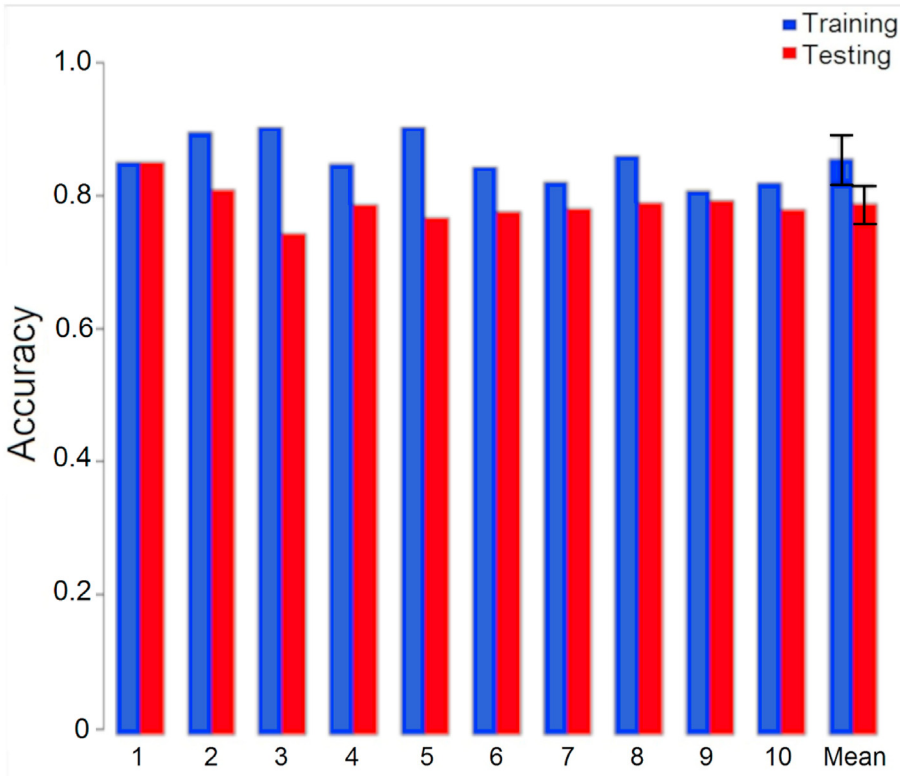

Fig. 6. CNN model accuracy assessed during 10 repeated cross-validation runs processing randomly selected image sets (n = 3723, 90% used for training, 10% for testing). Left columns represent the accuracy obtained for the training sets and right columns represent the accuracy obtained for the testing sets. The last two columns indicate the mean accuracy ± the standard deviation obtained within the 10 cross-validation runs.

2019; Maximenko et al., 2019). Remote sensing algorithms for beach monitoring are more advanced than those available for the sea surface, mainly because georeferenced orthomosaics are more easily produced from the overlapped photographs of beaches, where many reference points can be used for calibration (*e.g.*, trees, shrubs, plant logs). Furthermore, beach monitoring is less affected by environmental conditions such as perturbations on the sea surface caused by wind or sun glint, and the risk of losing unmanned vehicles is lower when flying over the land than over the sea surface (Fallati et al., 2019; Gonçalves et al., 2020b; Martin et al., 2018; Merlino et al., 2020). Conversely, monitoring FMML through remote sensing is further challenged by bright elements on the marine surface (*e.g.*, white caps, foam, waves, sun glint), cloud shadows (Dierssen and Garaba, 2020; Garaba and Dierssen, 2018; Matthews et al., 2017; Maximenko et al., 2019) and the fact that floating items can often be partially submerged in the water column (Van Sebille et al., 2020).

Machine learning algorithms have been used to automate marine litter recognition in aerial imagery, using, for instance, random forest (Gonçalves et al., 2020a, 2020b; Martin et al., 2018) or deep learning approaches (Fallati et al., 2019; Gonçalves et al., 2020b; Kako et al., 2020; Kylili et al., 2019). The main advantage of deep learning algorithms compared to their predecessors (*e.g.*, SVM, random forest, Multiple Regression) is that they can automatically identify the important features of an image without any human supervision, which makes them less time-demanding.

CNNs are the most popular deep learning architectures, inspired by the biological resemblance between the connectivity pattern of neurons and the organization of the animal visual cortex (Shridhar et al., 2019). Their effectiveness to identify images with hidden complex patterns (*e.g.*, Gonçalves et al., 2020b; Kylili et al., 2019) brought a raising interest on CNNs algorithms, which have been recently used for the automatic detection of litter, mainly on beaches (Fallati et al., 2019; Gonçalves et al., 2020b), but also on the water surface (Jakovljevic et al., 2020; Kylili et al., 2019) (Table 2).

Studies to automatically detect marine litter in aerial imagery of beaches were conducted by Fallati et al. (2019) and Gonçalves et al. (2020b), who used a DJI Phantom 4 drone equipped with an RGB high resolution camera for the purpose, and developed a deep learning software for the automatic detection of litter. While the former authors reported a similar accuracy (0.95) to that obtained in the current study (0.85) for the training set, their F1-score for the testing set was lower (Table 2), probably due to the high detection of false negatives and false positives due to footprints and shadows on the beaches. Gonçalves et al. (2020b) also reported a lower F1-score than that obtained in the current study (Table 2), mainly due to the detection of many false positives. The main difficulties faced by the CNN model developed by these authors were related to the identification of litter items trapped among natural wood and dune vegetation.

Suitable algorithms that could deal with these environmental variables have still to be developed to improve the efficiency of new remote sensing technologies for routine beach monitoring. However, the challenges posed by the detection of floating litter over the marine surface are even more difficult to cope.

A deep learning algorithm to classify plastic litter in images from the water surface was recently developed by Jakovljevic et al. (2020), who used a DJI Mavic pro equipped with an RGB camera to take images of the surface of enclosed bodies of water with ground sampling distances similar to those used in the current study (0.4–3.0 cm vs 0.6–3.6 cm pixel^{-1}, respectively). The authors also deployed "positive controls" (plastic bottles, ropes, and

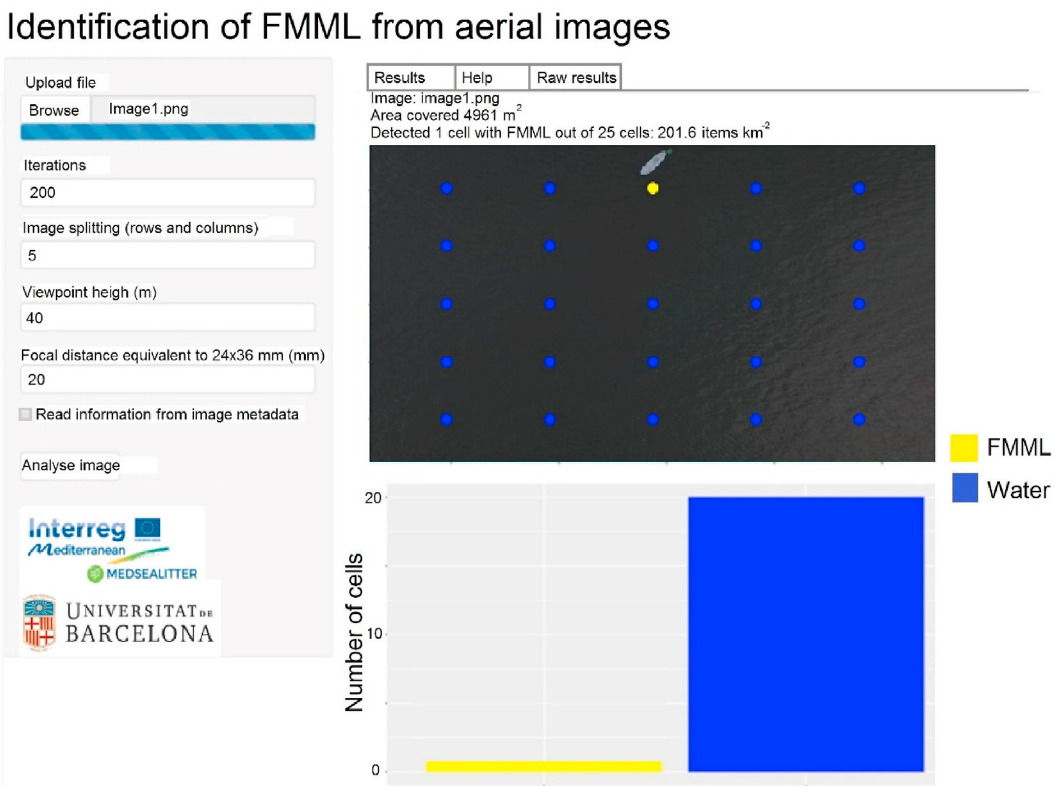

Fig. 7. Web-oriented application, named MARLIT, developed to detect interactively FMML in aerial images, based on the Shiny package. The MARLIT application can be downloaded from https://github.com/amonleong/MARLIT. On the left side of the app the user is able to: (1) browse and upload images, and specify: (2) the number of iterations used for the analysis, (3) the number of rows and columns for the image splitting, (4) the viewpoint height, and (5) the focal distance. Parameters for (2) and (3) should be at least 150 and 5 (to split the image into 25 cells), respectively. Information for (4) and (5) can be extracted directly from the image metadata. On the right side of the app, the panel shows the output of the analysis, including the image name, the area covered by the image, and the density of FMML detected. The above panel shows the image with its cells classified as containing FMML (yellow dots) and not containing FMML (blue dots); the bottom panel shows a histogram summarizing the number of cells containing and not containing FMML. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

polystyrene) on the water surface to train their model, reporting similar F1-scores to those obtained in the current study (Table 2), and subsequently tested the CNN algorithm in an independent scenario, obtaining F1-scores for plastic and “maybe plastic” of 0.78 and 0.43, respectively. The low F1-score for “maybe plastic” was due to the confusion of this category with those of “water” and “plastic”. However, although the methodology of Jakovljevic et al. (2020) is very similar to that applied here, their study was located in enclosed bodies of water (lakes), and thus some adverse elements (waves, foam, white caps) that may impair the detection of litter in the marine environment did not affect the performance of their algorithm.

Studies about automatic FMML detection in aerial RGB imagery are scarce: to the best of our knowledge, only Kylili et al. (2019) developed a CNN model for the automatic detection of FMML. The training and testing accuracies of the algorithms developed by these authors were higher than those obtained in the current study (Table 2). However, the images they used to test and train the model were taken from only a few meters above the sea surface, as their aim was the implementation of the model on a prototype device installed onboard marine vessels. Moreover, they used more images (9600) than those used here to train the CNN model, which may have been determinant to the higher level of accuracy attained (Sun et al., 2017).

The CNN algorithms presented in the current study were adapted from those currently available with the objective to streamline the process of FMML detection in images taken by aerial platforms (*e.g.*, drones, aircraft), which is highly time-demanding when performed by different trained scientists. As routine monitoring of FMML density, distribution, and trends is strongly recommended by national and international regulations, any improvement that would increase its efficiency and guarantee the consistency of results is highly valuable. Moreover, the development of user-friendly web-oriented applications such as MARLIT, the proof of concept presented here, may be of great interest for their implementation during regular monitoring of Marine Protected Areas, coastal areas, or even large oceanic areas. The current functionality of the application allows the user to upload aerial images and to get results in terms of presence and density of FMML and, if geo-referenced images are used, it could already provide an approximation of the presence of floating litter in a given marine area. The implementation of the CNN models developed in this study through a web-oriented application is a further step towards the automation of FMML detection and provides a useful approach to standardize FMML monitoring through any aerial platform (*e.g.*, a drone, or a small aircraft) equipped with remote sensing devices. It could be useful to classify high amounts of images as containing FMML or not, which in turn could help to identify potential areas of aggregation of litter at sea.

However, future improvements of the algorithm and the MARLIT application are needed to allow identifying the size, colour and type of FMML, which are relevant information for planning well-targeted policy and mitigation measures (GESAMP, 2019). The collaboration with other researchers to share data sets of sea surface aerial imagery would also facilitate the improvement of the current model accuracy. As well, further research is needed to allow implementing the application directly to remote sensing devices for the real-time inspection of the marine surface during aerial surveys.

5. Conclusions

In this paper, we propose the use of CNN-based deep learning models connected to a web-oriented application to process aerial images for the automatic detection and quantification of FMML. Its installation in remote sensing devices, such as RGB cameras mounted on aerial platforms, would allow streamlining the monitoring of FMML over marine areas at any geographical scale. Further research is needed to improve the current automated algorithms by increasing the number of images used for training (and thus improving detection accuracy), and to implement the application directly on remote sensing devices. Effective and feasible automated methods to monitor FMML could complement or replace the traditional methods for marine monitoring, significantly improving the quality of results.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.116490.

Credit author statement

Odei Garcia-Garin: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization **Toni Monleón-Getino**: Methodology, Validation, Writing – review & editing, Supervision **Pere López-Brosa**: Methodology, Validation, Writing – review & editing **Asunción Borrell**: Resources, Supervision, Project administration, Funding acquisition **Alex Aguilar**: Resources, Supervision, Project administration, Funding acquisition **Ricardo Borja-Robalino**: Methodology, Validation, Writing – review & editing **Luis Cardona**: Resources, Funding acquisition **Morgana Vighi**: Conceptualization, Methodology, Resources, Writing – review & editing, Supervision

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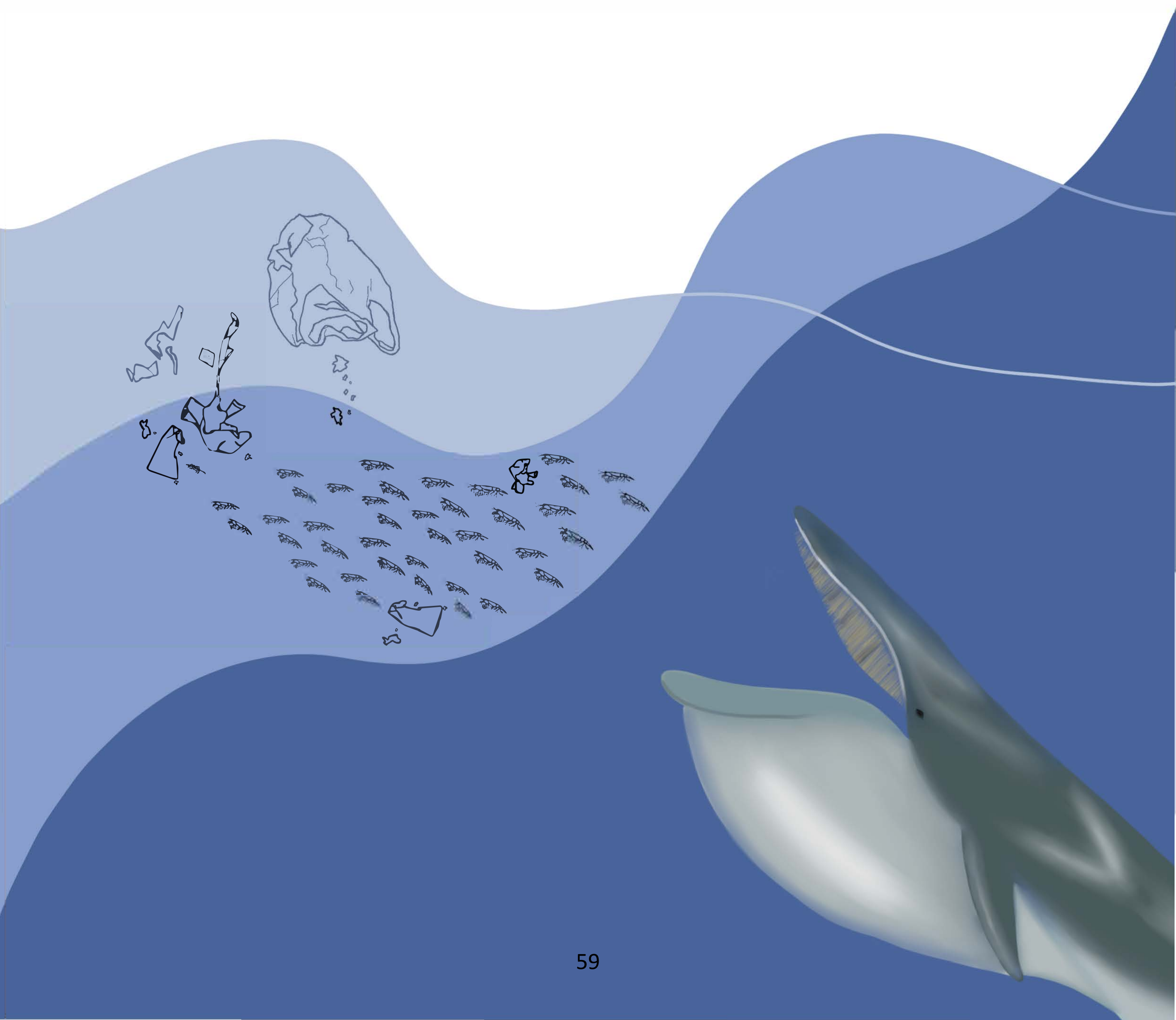
2. MICROPLÁSTICOS



2.1. *Boops boops* as a bioindicator of microplastic pollution along the Spanish Catalan coast

Autores: Odei Garcia-Garin[1], Morgana Vighi[1], Alex Aguilar[1], Catherine Tsangaris[2], Nikoletta Digka[2], Helen Kaberi[2], Asunción Borrell[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain
[3]Institute of Oceanography, Hellenic Centre for Marine Research, Athens, Greece

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Abstract:
Microplastic pollution is a growing cause of concern for the marine environment, particularly in the Mediterranean Sea, which is considered to be one of the most polluted seas worldwide. In this study, the gastrointestinal tracts of 102 bogues (*Boops boops*), sampled from three areas off the Catalan coast (Spain) subject to different degrees of industrialization, were analysed to assess microplastic ingestion and thus estimate local levels of microplastic pollution. Microplastics were detected in 46% of samples analysed. As expected, the abundance and frequency of occurrence of ingested microplastics were higher off the most anthropized area of Barcelona. The majority of ingested microplastics were blue fragments ranging 0.1–0.5 mm, and the most common polymer type was polypropylene. The results of this study indicate the area off Barcelona as a possible area of concentration for microplastics, further supporting the use of *B. boops* as a bioindicator to assess microplastic pollution.

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Odei Garcia-Garin[a,*], Morgana Vighi[a], Alex Aguilar[a], Catherine Tsangaris[b], Nikoletta Digka[b], Helen Kaberi[b], Asunción Borrell[a]

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

[b] *Institute of Oceanography, Hellenic Centre for Marine Research, Athens, Greece*

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ABSTRACT

Microplastic pollution is a growing cause of concern for the marine environment, particularly in the Mediterranean Sea, which is considered to be one of the most polluted seas worldwide. In this study, the gastrointestinal tracts of 102 bogues (*Boops boops*), sampled from three areas off the Catalan coast (Spain) subject to different degrees of industrialization, were analysed to assess microplastic ingestion and thus estimate local levels of microplastic pollution. Microplastics were detected in 46% of samples analysed. As expected, the abundance and frequency of occurrence of ingested microplastics were higher off the most anthropized area of Barcelona. The majority of ingested microplastics were blue fragments ranging 0.1–0.5 mm, and the most common polymer type was polypropylene. The results of this study indicate the area off Barcelona as a possible area of concentration for microplastics, further supporting the use of *B. boops* as a bioindicator to assess microplastic pollution.

1. Introduction

The presence of marine litter has been reported in all marine compartments of seas and oceans worldwide (Cózar et al., 2014; Alomar et al., 2016). The largest component of marine litter is represented by artificial polymers, *i.e.,* plastics (Geyer et al., 2017). Large plastic items that enter the sea are gradually broken into small pieces by the mechanical erosion caused by winds and waves, photodegradation, and biodegradation (Barnes et al., 2009; Thompson et al., 2004), and gradually become microplastics *i.e.,* plastic items smaller than 5 mm in size (Arthur et al., 2009). Apart from these, microplastics can be of primary origin, which include the microbeads used in cosmetics and personal care products, capsules, textile microfibres, or virgin pellets used for manufacturing larger plastic items. Once in the sea, microplastics are driven by oceanic currents, travel long distances due to their buoyancy and durability (Eriksen et al., 2014), and they represent a considerable portion of the litter found in marine waters (de Haan et al., 2019). Recent studies estimated that 5 trillion microplastics are currently floating in the world's oceans and that the concentration of plastic particles floating in the surface waters of the Mediterranean Sea is 890,000 particles km^{-2} (Eriksen et al., 2014).

Microplastics may pose a threat to the marine environment (Rezania et al., 2018). Marine species at all levels of the trophic chain, including zooplankton (*e.g.,* Cole et al., 2014), worms (Wright et al., 2013), shellfish (*e.g.,* Digka et al., 2018), fish (*e.g.,* Bellas et al., 2016), seabirds (Codina-García et al., 2013), sharks (Fossi et al., 2014) and cetaceans (Fossi et al., 2016) have been reported to ingest microplastics. Despite evidence of the translocation of microplastics from the gastrointestinal tract to other tissues, *i.e.,* the presence of microplastics in the hepatic tissue of the mullet (*Mugil cephalus*) under laboratory conditions (Avio et al., 2015) and in eviscerated flesh of four commonly consumed dried fish species (Karami et al., 2017), related adverse effects in wild organisms are still lacking (Avio et al., 2015). Furthermore, although microplastics are chemically inert, the organic compounds used as plasticizers to improve the properties of plastics might produce adverse effects in some marine species, including alterations in the endocrine system and reproductive capacity (Lithner et al., 2011). Moreover, persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and dichlorodiphenyltrichloroethane (DDT) may be adsorbed and accumulated on post-consumed microplastics, increasing their toxic potential effects (Rios et al., 2007).

Different methods are used to assess the extent of microplastic pollution in the sea and thus estimate its potential risk for marine fauna.

* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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Manta trawl nets are employed to assess the density of microplastics floating in the water column (*e.g.*, de Haan et al., 2019), while analyses of sediment samples are used to determine microplastic densities in the ocean floor and beaches (*e.g.*, Van Cauwenberghe et al., 2014; Alomar et al., 2016). Bioindicator species have also been proven particularly effective in assessing the microplastics levels in the biota (Fossi et al., 2018) and thus, potentially, their environmental concentrations. The EU Marine Strategy Framework Directive (MSFD) monitoring guidelines for the Mediterranean Sea indicate the analysis of the fish gastrointestinal tract (GI) as a viable method to assess microplastic pollution (Galgani et al., 2013). Among the possible fish species proposed, the bogue (*Boops boops*; Linneaus, 1758) stands out as a suitable bioindicator due to its ubiquitous distribution in the Mediterranean, the small size of its gut, and the high frequency of occurrence of microplastics in its digestive tract (Bray et al., 2019). In addition, as this species feeds on different types of bottoms including sand, mud, rocks and seagrass beds, performing vertical migrations at depths ranging from 0 to 350 m, it can be representative of several marine compartments (El-Haweet et al., 2005). Finally, its commercial value across the Mediterranean facilitates sample collection in local markets and thus further supports the use of the bogue as a commonly agreed upon bioindicator (Bray et al., 2019).

In the present study, the GI content of *B. boops* was analysed to assess the levels of microplastic ingestion in three differently urbanized and industrialized areas off the Spanish coast of the Mediterranean Sea: (1) the area off Barcelona, affected by several anthropogenic activities producing marine litter inputs, such as industrial outfalls, beach tourism, fishing, aquaculture and shipping; (2) the area off the small town of Blanes, characterized by local tourism and fishing activities; and (3) the area off Cap de Creus, a marine protected area (MPA), subject to heavy dominant winds and currents, where fishing and tourism are regulated. The aim of the study was to identify any differences in microplastic levels among the three areas and validate the use of the bogue as a bioindicator for microplastic pollution.

2. Materials and methods

2.1. Study area and sampling

A total of 102 bogues were collected during spring 2018 in three different areas of the Spanish Catalan coast (34 specimens per area), selected according to a gradient of industrialization and urbanization: 1) a highly anthropized area, located off the city of Barcelona; 2) an intermediate-anthropized area, near the town of Blanes; 3) an MPA, off Cap de Creus (Fig. 1). Fish were caught by local fishermen using trawling (22 individuals from Cap de Creus and 13 from Barcelona), purse seine (34 individuals from Blanes and 21 from Barcelona) and trammel nets (12 individuals from Cap de Creus) in areas located between 3 and 9.5 km from the coastline, at depths ranging between 22 and 90 m. After collection, fish were stored at −20 °C. Total length and total wet weight were measured for each fish (Table S1).

2.2. Extraction of microplastics

Fish were defrosted at 5 °C before dissection. The fish GI were dissected and weighed (wet weight, GIWW). To eliminate organic matter and enable detection of microplastics, samples were digested with hydrogen peroxide according to the protocol defined within the MEDSEALITTER project (MEDSEALITTER consortium, 2019). The GI content of each individual was placed into a glass beaker in 1:20 (w/v) H_2O_2 (15% H_2O_2, Chem-Lab, Germany) and heated on a hot plate at 55–65 °C until H_2O_2 evaporation. Aliquots of 10 ml H_2O_2 were added gradually to the beakers until all the organic matter was digested (the digestion process taking between 48 and 96 h). Samples were then diluted with 50 ml Milli-Q and vacuum-filtered on fibreglass filters (pore size 1.2 μm, Whatman, GE Healthcare, UK), which were dried at room temperature for 24 h and subsequently stored in Petri dishes.

2.3. Microplastic detection and quantification

Filters were examined under a stereomicroscope (Olympus, SZE and SZX7), and the microplastics detected were photographed using a digital camera (Luminera) and the INFINITY ANALYZE software. Items were counted and classified in four categories according to maximum length (< 0.1, 0.1–0.5, 0.5–1.0, 1.0–5.0 mm), colour, and type (fragment, fibre and granule). Average microplastic abundance was expressed as a) average number of microplastic items per individual considering the total number of examined individuals, b) average number of microplastic items per individual considering only individuals containing microplastics and c) average number of microplastic items per gram GIWW, considering only individuals containing microplastics. The frequency of occurrence of ingested microplastics was calculated as the percentage of the individuals containing microplastics out of the total number of sampled individuals.

2.4. FT-IR analysis

Fourier-transform infrared spectroscopy (FT-IR) was used in microplastic items larger than 300 μm to identify the type of synthetic polymer. FT-IR analysis was carried out with an Agilent Cary 630 FT-IR spectrometer using a self-generated polymer library. The confidence level for the comparison of the sample spectrum to that of the self-generated library database was set up to 80% (Digka et al., 2018). A minimum of 10% of the microplastics detected in the bogues GIs were analysed by FT-IR, as recommended by the marine litter monitoring guidelines provided by the MSFD technical group on marine litter (Galgani et al., 2013).

2.5. Contamination precautions and quality control

To prevent contamination throughout the analysis, the researchers performing the analyses wore white coats, and air currents were reduced to a minimum. All glass beakers were rinsed with purified water and fish samples were covered with aluminium foil during digestion. A glove bag was used for sample rinsing and filtration. Filters were protected with glass lids during stereoscope observation. Procedural blank samples were used during all steps, and items similar to those found in blank samples were excluded from statistical analyses, as they were considered airborne contamination.

2.6. Statistical analysis

Standardized data exploration techniques were used to identify outliers and possible collinearity between the physiological and spatial terms (Zuur et al., 2010). Microplastic abundance (calculated as in a), i.e., number of items per individual) in *B. boops* was modelled using GLMs (generalized linear models) with a negative binomial error distribution to account for overdispersion. Models were fitted with different combinations of the following explanatory variables: the level of anthropogenic impacts, categorized as low (MPA), medium (Blanes), high (Barcelona); the depth of the fishing area; the distance between the fishing area and the coastline, calculated using the measuring tool from Qgis (QGIS Development Team, 2018); the fishing method (trawling, purse seine and trammel nets); and the Fulton's condition factor, calculated as: $K = 100 * (\text{weight}/\text{total length}^3)$ (Froese, 2006). The information-theoretic approach was used for model selection (Burnham and Anderson, 2002) and models were compared using the AIC (Akaike's Information Criterion) (Akaike, 1974).

A Tukey HSD test was performed to compare microplastic abundance (a) in the three sampling areas. Correlations between the number and size of the ingested microplastics, and the fish body length, weight and GIWW were tested using Spearman's rank correlations. Types of

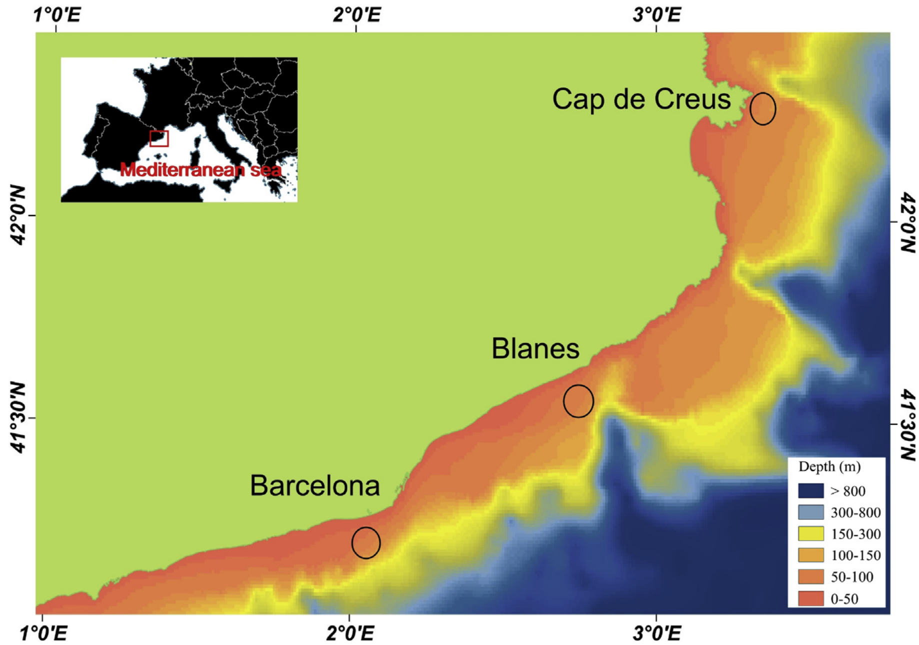

Fig. 1. Study area showing the three sampling areas: Barcelona, Blanes and Cap de Creus MPA.

ingested microplastics (shapes, class sizes and colours) were compared using the Pearson's Chi-squared test. The significance level was set at $p < 0.05$. Calculations were carried out within the programming environment R (R Core Team, 2014).

3. Results

3.1. Microplastic quantification for each area

In total, 46% of the fish had microplastics in their GI tracts. Microplastic abundance (a) ranged from 0 to 6 items per individual and the frequency of occurrence of ingested microplastics was higher in samples from the area off Barcelona (65%) than in those from the areas off Blanes and Cap de Creus (35% and 38%, respectively) (Table 1).

A total of 32 different GLMs were fitted from the combination of the 6 variables plus the Depth*Coast interaction (Table 2). The model with the lowest AIC score was that including the level of anthropogenic impacts and the distance to the coastline (M19, AIC = 243; Table 2), suggesting that higher ingestion rates of microplastics occur in locations near the coastline and with high anthropogenic impacts (Table 3). Accordingly, results from the Tuckey HSD test highlighted significant differences in microplastic abundance between the area off Barcelona and the other two areas (Table 4), while the difference in microplastic abundance between the area off Blanes and that off Cap de Creus was very small (0.50 ± 0.14 and 0.53 ± 0.14, respectively; Table 1). GLMs taking into account depth, fishing method and condition factor were not significant (Table 2).

In the bogues sampled off Barcelona and Blanes, the number of ingested microplastics showed a significant negative correlation with the fish body length (Spearman's r, S = 10,397, $\rho = -0.59$, $p < 0.001$ and S = 8,901, $\rho = -0.36$, $p < 0.05$; respectively) and the fish weight (Spearman's r, S = 88,724, $\rho = -0.62$, $p < 0.001$ and S = 14,842, $\rho = -0.50$, $p = 0.001$; respectively). Conversely, none of these correlations were significant in samples from the Cap de Creus MPA (Spearman's r, S = 6,309, $\rho = 0.04$, $p = 0.84$ and S = 8,979, $\rho = 0.09$, $p = 0.58$) (Fig. 2).

No correlation was found between the number of ingested microplastics and GIWW in samples from Blanes and the Cap de Creus MPA (Spearman's r, S = 7,911, $\rho = -0.21$, $p = 0.24$, and S = 6,774, $\rho = -0.36$, $p = 0.84$; respectively), while the number of ingested microplastics showed a negative correlation with GIWW in samples from Barcelona (Spearman's r, S = 10,377, $\rho = -0.59$, $p < 0.001$). Finally, no correlations were found between the microplastic size and the fish body length, weight or GIWW (Spearman's r, $p > 0.05$).

Table 1
Biological parameters, frequency of occurrence and abundance of ingested microplastics (MP) in *B. boops* from the three sampling areas.

Area	Barcelona	Blanes	Cap de Creus MPA
Number of individuals examined	34	34	34
Mean fish length (cm)	19.41 ± 2.81	19.86 ± 1.11	23.97 ± 3.93
Mean fish weight (g)	74.43 ± 28.69	103.92 ± 18.05	178.10 ± 111.65
Fulton's condition factor (K)	0.99 ± 0.11	1.32 ± 0.12	1.17 ± 0.17
Mean GIWW (g)	4.98 ± 2.26	8.17 ± 2.04	9.81 ± 3.66
Number of individuals containing MP	22	12	13
MP frequency of occurrence (%)	64.71	35.29	38.24
MP number	57	17	18
MP longest dimension length range (μm)	50–2960	66–3300	88–4700
MP abundance (mean ± SD)			
a) Number of items per individual in all individuals examined	1.68 ± 0.31^{a}	0.50 ± 0.14^{b}	0.53 ± 0.14^{c}
b) Number of items per individual in individuals containing MP	2.59 ± 0.35	1.42 ± 0.23	1.38 ± 0.18
c) Number of items per gram weight in individuals containing MP	0.83 ± 0.15	0.20 ± 0.05	0.16 ± 0.02

[a, b, c] Indicate significant differences between fish sampling areas (Tuckey HSD test).

Table 2
Results from the GLMs fitted with a negative binomial error distribution and ranked by Akaike Information Criterion (AIC) for microplastic abundance (a) in *B. boops*. Explanatory variables included in the models: level of anthropogenic impacts (low, medium and high), depth (m), coastline distance (km), fishing method (trawling, purse seine and trammel nets) and condition factor (Fulton's K). The best-fit model is shown in bold.

	Model	AIC
M1	Level of anthropogenic impacts + Coast * Depth + K + Method	251
M2	Level of anthropogenic impacts + Coast + Depth + K + Method	251
M3	Level of anthropogenic impacts + Coast + Depth + K	247
M4	Level of anthropogenic impacts + Coast + Depth + Method	249
M5	Level of anthropogenic impacts + Coast + K + Method	249
M6	Level of anthropogenic impacts + Depth + K + Method	259
M7	Coast + Depth + K + Method	276
M8	Level of anthropogenic impacts + Coast + Depth	245
M9	Level of anthropogenic impacts + Coast + K	245
M10	Level of anthropogenic impacts + Depth + K	260
M11	Level of anthropogenic impacts + K + Method	257
M12	Level of anthropogenic impacts + Depth + Method	257
M13	Level of anthropogenic impacts + Coast + Method	247
M14	Depth + K + Method	275
M15	Coast + K + Method	274
M16	Coast + Depth + Method	274
M17	Coast + Depth + K	274
M18	Level of anthropogenic impacts + Depth	260
M19	**Level of anthropogenic impacts + Coast**	**243**
M20	Level of anthropogenic impacts + K	259
M21	Depth + Method	276
M22	K + Method	274
M23	Coast + Method	273
M24	Level of anthropogenic impacts + Method	255
M25	K + Depth	272
M26	Coast + K	273
M27	Coast + Depth	273
M28	Level of anthropogenic impacts	259
M29	Method	274
M30	Coast	274
M31	K	271
M32	Depth	272

3.2. Microplastic characterization (shape, size, colour and polymer type)

The proportion of shape, size class and colour categories did not differ among areas (Pearson's Chi-squared test, $p > 0.05$). The majority of ingested microplastics in the three areas were fragments of different colours and sizes (Fig. 3). The most common size class was 0.1–0.5 mm, found in the samples from all areas (Fig. 3 B), and the most common colour was blue in the samples from Barcelona and Blanes and black in the samples from Cap de Creus MPA (Fig. 3C).

Considering the microplastics analysed by FT-IR ($n = 9$), polypropylene was the most common polymer type (56%), followed by polyethylene (33%) and polystyrene (11%). Examples of microplastics found in the fish GI with the corresponding FT-IR spectra are shown in Fig. 4.

4. Discussion

In this study, the ingestion of microplastics was investigated in bogue samples to assess the levels of microplastic pollution in three areas off the Catalan coast and validate the use of this species as a bioindicator for microplastic pollution. The use of bioindicator species is strongly recommended by the MSFD and other monitoring programmes (e.g. UNEP/MAP) to increase the knowledge on the extent of marine litter pollution and its impacts on marine species. Previous studies made using the same species as a bioindicator detected similar microplastic occurrence levels in the Balearic Islands of Mallorca and Ibiza (Mediterranean Sea) (Nadal et al., 2016). The occurrence of microplastic found by these authors in the full stomach and intestine of the 337 bogues analysed was 68%. However, only 9% of the 32 bogues sampled by Neves et al. (2015) in the North Atlantic, off the Portuguese coast, had microplastics in their digestive tracts, indicating a spatial variability in the levels of microplastic ingested by the bogues that reflects local levels of microplastics in the sea.

Table 4
Summary of the results from the Tuckey HSD multiple comparisons of means for the factor “level of anthropogenic impacts” (categorized in: Low (“L”), Medium (“M”), High (“H”)).

Linear hypotheses	Coefficient estimate	Standard error	Z value	Pr(> |z|)
L – H = = 0	−3.36	0.61	−5.48	< 0.001
M – H = = 0	−1.87	0.33	−5.69	< 0.001
M – L = = 0	1.49	0.54	2.75	0.02

4.1. Microplastic quantification

Significant differences were detected in the levels of microplastics ingested by *B. boops* in the three areas. As expected, the results of microplastic quantification indicated that bogues sampled from the most anthropized area off Barcelona presented the highest abundance and frequency of occurrence of ingested microplastics. Our results are consistent with those obtained by Bellas et al. (2016), who analysed microplastic ingestion by the demersal fish species *Mullus barbatus* in three areas off the Spanish Mediterranean coast and found the highest microplastic occurrence (33.3%) in the samples from the area off Barcelona.

Barcelona is located between two rivers, the Besòs and the Llobregat, and hosts a population of 1.6 million people (Instituto Nacional de Estadística, http://www.ine.es/welcome.shtml), a number of large industries, one of the most important commercial and tourist ports of the Mediterranean coast, and a large airport. Liubartseva et al. (2018) identify Barcelona as the second city of the Mediterranean Sea in terms of estimated inputs of plastic marine debris, with a total contribution of 1800 tons per year. Dominant marine currents along the Catalan coast follow a pattern from north to south parallel to the coast. They originate from the 30-km wide mesoscale Northern Current, which flows cyclonically along the continental slope from the Gulf of Genova to the southern Gulf of Valencia (Font et al., 1995). Indeed, urbanization has been reported to have a major influence on microplastic ingestion by fish (Peters and Bratton, 2016), and locations where currents converge accumulate marine litter and therefore marine biota more frequently ingest microplastics (Moore et al., 2001). Due to all these factors, bogues sampled in the marine area off Barcelona are exposed to higher microplastic concentrations than those occurring in other areas along the Catalan coast.

The amounts of microplastics found in the GI tracts of the bogues

Table 3
Summary of the results from the best-fit GLM, fitted with the variables “level of anthropogenic impacts” and “distance to the coastline” (M19).

Term	Coefficient estimate	Standard error	Z value	Pr(> |z|)
Intercept	5.20	1.10	4.73	< 0.001
Level of anthropogenic impacts (Low)	−3.36	0.61	−5.48	< 0.001
Level of anthropogenic impacts (Medium)	−1.87	0.33	−5.69	< 0.001
Coast	−0.62	0.15	−4.20	< 0.001

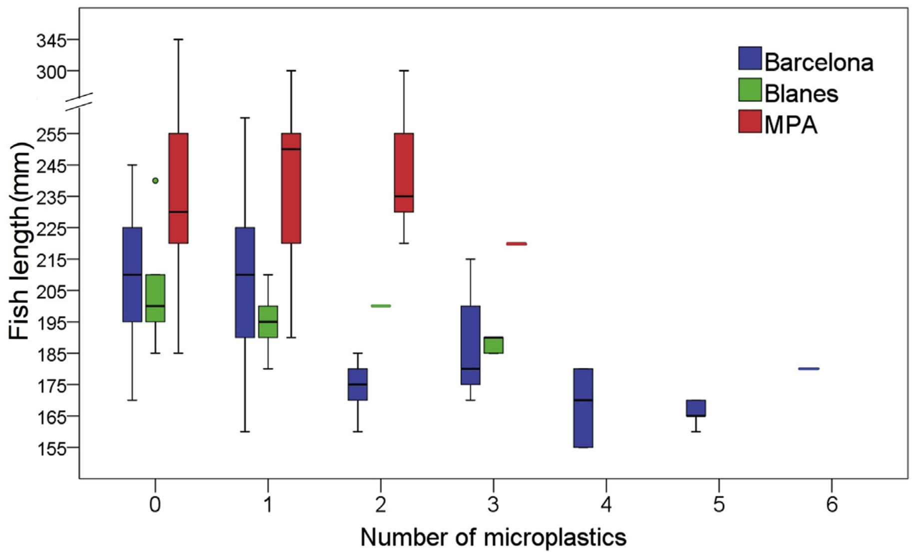

Fig. 2. Box plot showing the relationship between the bogues body length and the number of microplastics ingested. The central line indicates the median fish length for each area and number of microplastics; the edges of the box indicate the 25th and 75th percentiles; whiskers extend to extreme data points not considered outliers, and outliers are plotted individually as circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sampled in the area off Blanes and in the Cap de Creus MPA were similar, and the average frequency of occurrence in both areas was consistent with the value of 37.5% found by Rios-fuster et al. (2019) in *B. boops* from southern Spain. The same authors reported similar values of microplastic occurrence (≈30%) also in samples of *Sardina pilchardus* from Blanes and *Trachurus mediterraneus* and *Engraulis encrasicolos* from Cap de Creus MPA. Although lower abundance and frequency of occurrence might be expected in the marine protected area, consistently with our results, Nadal et al. (2016) also found high frequencies of microplastic occurrence in bogues sampled from Espardell, an island inside the MPA Ses Salines (Eivissa, Spain). These discrepancies indicate that microplastic presence in the sea must be interpreted from a wider perspective, evaluating levels of industrialization and urbanization in the proximity, but also the influence of seasonal currents, river discharges, wastewater treatments, rainfall, and tourism fluxes. The Cap de Creus MPA is very popular among international tourists due to its high natural and cultural values, and despite its high level of protection and preservation, high amounts of litter are generated on the land that may accidentally enter the sea. Furthermore, the dominant pattern of winds and currents may also generate local areas of microplastic accumulation during certain periods of the year.

Results obtained from the best-fit model showed that bogues ingest higher rates of microplastics closer to the coastline. This result is

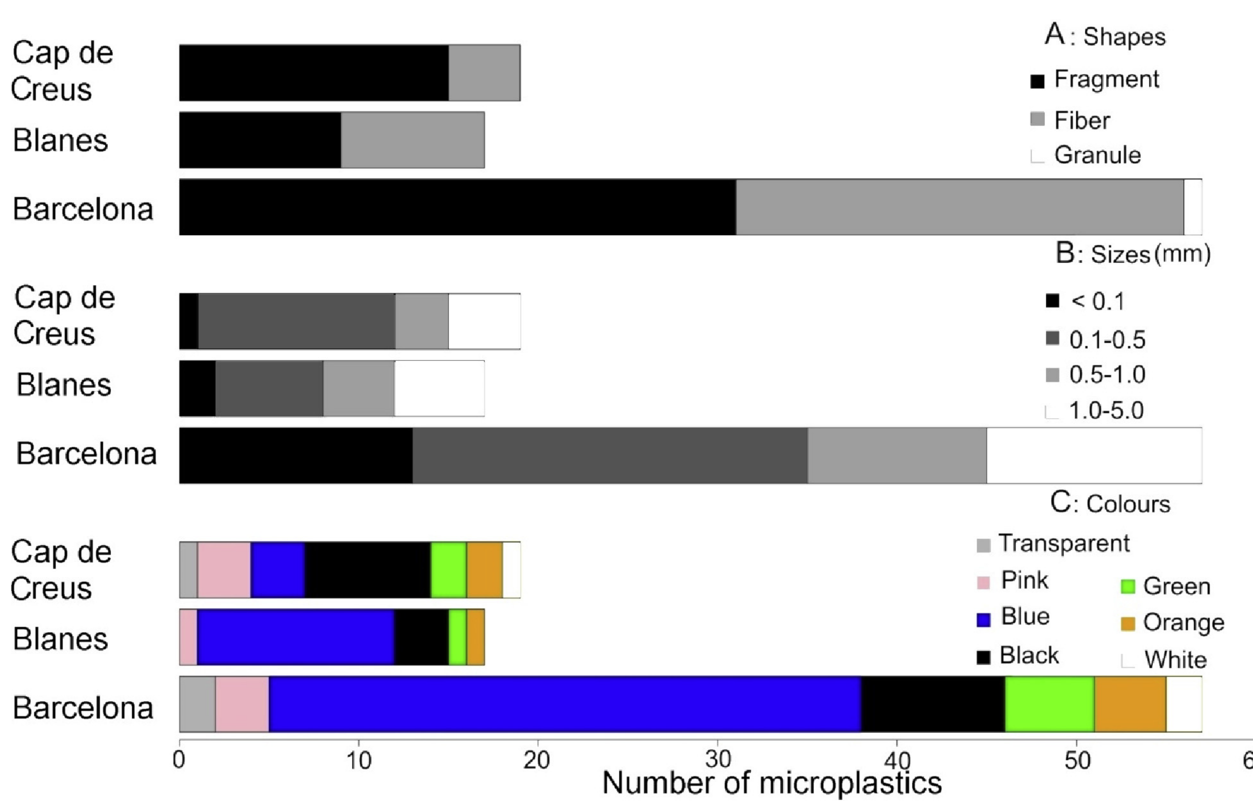

Fig. 3. Shape (A), size (B) and colour (C) of microplastics detected in *B. boops* from the three sampling areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

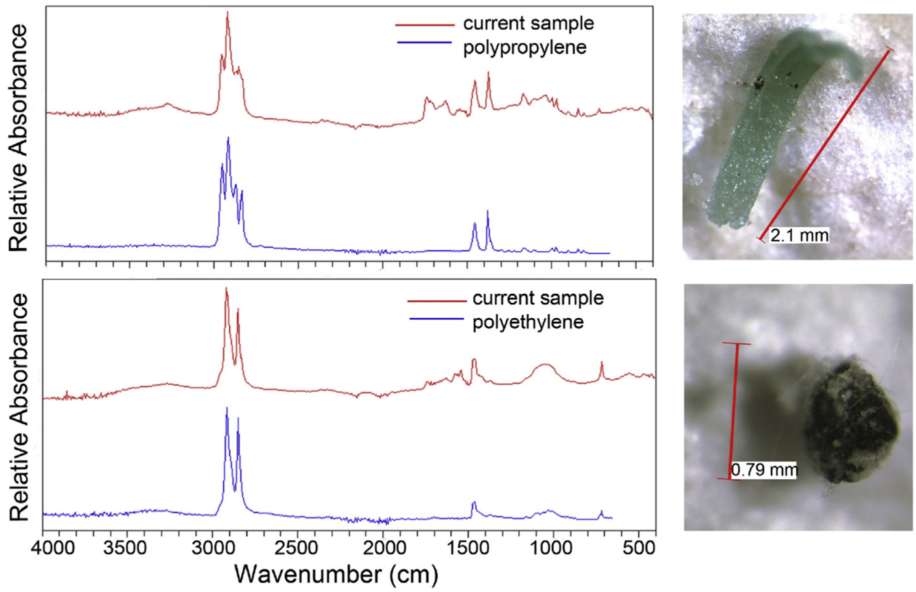

Fig. 4. Examples of microplastics found in fish gastrointestinal tract with relative Fourier-transform infrared spectroscopy spectra (level of certainty of 82 and 95% for the first and second microplastic item spectra, respectively).

consistent with those obtained by Rios-fuster et al. (2019), and confirms the hypothesis that the greatest overlap between microplastics and marine fauna occurs in coastal waters (Clark et al., 2016), as higher concentrations of litter are often found in proximity of densely populated urban centres, touristic areas and shipping routes (Suaria et al., 2014).

The abundance of ingested microplastics was inversely correlated with body length and weight in the bogues from Barcelona and Blanes but not in those from Cap de Creus MPA. Although similar studies show no effect of body length on microplastic ingestion occurrence in other fish species (*e.g.*, Foekema et al., 2013; Digka et al., 2018), some authors suggest that larger individuals are less likely to ingest microplastics (*e.g.*, Compa et al., 2018; Bessa et al., 2018), which may explain the higher abundance of microplastics in the GIs of the smaller individuals from Barcelona and Blanes. However, explanations for the discrepancy of the relationship between microplastics and body length between areas remain unknown, and it should be highlighted that the bogues from Cap de Creus were, on average, larger in size and weight, which likely had an effect on that relationship (Fig. 2). In addition, no correlation with the Fulton's condition factor (K) was found in the bogues sampled for this study, despite Compa et al. (2018) reported that individuals of *S. pilchardus* with lower condition factor ingested more microplastics than those individuals in better conditions. Although Compa et al. (2018) did not find any difference in the abundance of ingested microplastics between mature and immature individuals, microplastic ingestion rates could be also related with the fish developmental stages, as mature and immature individuals often show behavioural and feeding habits dissimilarities.

4.2. Microplastic characterization

Microplastics ingested by *B. boops* from the Catalan coast were primarily fragments (~60%) and secondly fibres (~40%) (Fig. 3A). Fragments are the result of the degradation of larger plastic items, while fibres are the most abundant component of primary microplastics in seas and oceans worldwide (Bessa et al., 2018). Our results revealed, proportionally, a smaller contribution of fragments and a larger contribution of fibres than those detected in fish of the Northern Ionian Sea by Digka et al. (2018), who reported approximately 80% fragments and 20% fibres, respectively, showing a similar order of prevalence. Conversely, other studies (e.g., Lusher et al., 2013; Bellas et al., 2016; Güven et al., 2017; Compa et al., 2018; Bessa et al., 2018) found a higher percentage of fibres than fragments in fish GIs. These contrasting results may be related to different sources and waste management strategies in the sampling areas, which could prevent or reduce the amounts of plastic items that reach the sea from land, brought by rivers or wind (Digka et al., 2018; Boucher and Friot, 2017).

In the present study, microplastics were classified into 4 size categories according to their largest dimensions. The main microplastic size class was 0.1–0.5 mm (Fig. 3B), supporting the role of indirect intake from microplastics ingested by prey (*i.e.,* zooplankton) as an important mechanism of microplastic ingestion in fish (Avio et al., 2017; Neves et al., 2015). However, future research is needed to improve knowledge regarding the mechanisms of microplastic ingestion by bogues (Nadal et al., 2016). In addition, Digka et al. (2018) also found that microplastics between 0.1 and 0.5 mm were the most prevalent in mussels and fish from the Adriatic Sea. However, microplastics < 0.1 mm may have been underestimated due to the reduced recovery rates for smaller particles (Avio et al., 2015).

The predominant colour of the microplastics ingested by bogues was blue (Fig. 3C), a result consistent with other studies (*e.g.*, Romeo et al., 2015; Güven et al., 2017; Peters et al., 2017; Compa et al., 2018; Digka et al., 2018). The prevalence of this colour may suggest that fish ingest microplastics regardless of their colour, as blue microplastics are not distinctively visible to fish (Peters and Bratton, 2016).

Finally, the most common polymer types detected in the litter ingested by *B. boops* were polypropylene, polyethylene and polystyrene. These results were expected because these three polymers are present in most plastic litter found in the water column worldwide (Suaria et al., 2016; Cózar et al., 2017). Polyethylene is used to manufacture plastic bags and bottles (Suaria et al., 2016; Cózar et al., 2017), which makes it the most abundant plastic in the world; polypropylene is highly abundant in bottle caps and packages (Suaria et al., 2016); and polystyrene is used widely for fishing boxes and other common containers. Consistently with our findings, polypropylene and polyethylene were also predominant in other studies of microplastic ingestion in fish from the Mediterranean Sea (Avio et al., 2017; Digka et al., 2018) and other European seas (Collard et al., 2017).

4.3. The use of bioindicators for marine litter monitoring in the international legislative framework

New international and EU directives are focusing on the reduction of waste and on the implementation of monitoring programs to assess the extent of marine litter pollution and its impacts in order to plan adequate mitigation measures. Among others, the Waste Directive (amending 2008/98/EC), the Packaging Directive (94/62/EC), the Plastic Carrier Bags Directive (2015/720/UE amending 94/62/EC), the Single Use Plastic Directive (2018/0172/EC) and the Directive on Port reception facilities for the delivery of waste from ships (directive COM (2018) 33) are addressing these issues. In addition, the UNEP/MAP Regional Plan for Marine litter Management in the Mediterranean (UNEP/MAP IG.21/9) highlights the urgent need to act against marine litter. From the UN Environment Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP), adopted in 2016, the use of bioindicator species for marine litter monitoring is clearly recommended by the Candidate Indicator 24: *Trends in the amount of litter ingested by or entangling marine organisms, focusing on selected mammals, marine birds, and marine turtles,* under Ecological Objective 10 (EO10). Moreover, the UNEP/MAP (Galgani, 2017) reported recently that bioindicator species are highly needed to monitor microplastics and marine litter in general. To comply with legal requirements and the urgent need to address the issues posed by marine litter, several studies focusing on microplastic ingestion are investigating suitable bioindicator species (Bray et al., 2019; Fossi et al., 2018). In this framework, furthermore, MSFD (Commission Decision, 2017/848) aims to achieve the Good Environmental Status, and it will be possible when we achieve the D10 criteria, which states: *Properties and quantities of marine litter do not cause harm to the coastal and marine environment*. Results from the present article provide a further support for the adoption of *B. boops* as a bioindicator species for marine litter (*i.e.,* the ever-increasing microplastics) monitoring.

5. Conclusions

Our results identify the area off Barcelona as a possible area of concentration for microplastics and further support the use of *B. boops* as bioindicator of microplastic pollution in the Mediterranean Sea, potentially reflecting both environmental microplastic loads and their main characteristics. In addition, the results from this study contribute to increasing the knowledge about levels of microplastic pollution in the Mediterranean, highlighting that highly anthropized areas can be potential hotspots for microplastic accumulation and thus ingestion by marine fauna. The assessment of microplastic levels and the identification of potential hotspots of microplastic accumulation and/or higher risk for marine fauna is a necessary requirement for planning targeted measures to reduce the potential risks related to marine litter.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.110648.

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2.2. Ingestion of synthetic particles by fin whales feeding off western Iceland in summer

Autores: Odei Garcia-Garin[1], Alex Aguilar[1], Morgana Vighi[1], Gísli A. Víkingsson[2], Valérie Chosson[2], Asunción Borrell[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain
[2]Marine and Freshwater Research Institute, Fornubuðir 5, 220, Hafnafj€orður, Iceland

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Abstract:
In this study we aim to assess the daily ingestion rates of synthetic particles by the fin whales (*Balaenoptera physalus*) that feed off the western coast of Iceland. To do so, we collected and analysed samples from the stomach content of 25 fin whales, consisting solely of northern krill (*Meganyctiphanes norvegic*a). The particles found consisted of fibres and fragments, mainly blue, black and red, with an average size of 1.2 ± 1.3 mm. To confirm the synthetic nature of these particles, we used Micro-Fourier Transform Infrared Spectroscopy and comparison with a polymer library. The mean concentration of synthetic particles in the krill samples found in the stomachs of whales was 0.057 particles per gram, a value much lower than that previously reported for particle uptake by krill. From this concentration in krill, we estimated that the daily intake of synthetic particles for the North Atlantic fin whale would be ranging from 38,646 ± 43,392 to 77,292 ± 86,784 particles per day. Although at this level it is not possible to assess the impact of synthetic particles and their associated chemicals on the North Atlantic fin whale population, concentrations of these contaminants are likely to increase in the future, potentially causing adverse effects on whales and other marine mammals.

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Ingestion of synthetic particles by fin whales feeding off western Iceland in summer

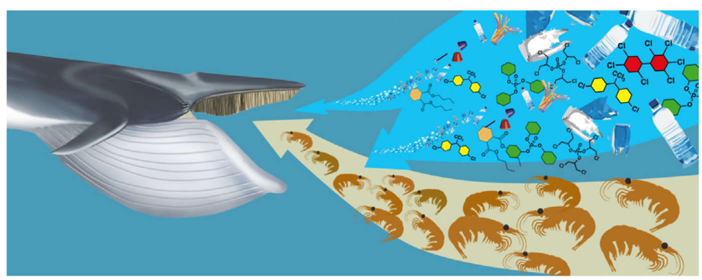
Odei Garcia-Garin [a], Alex Aguilar [a], Morgana Vighi [a], Gísli A. Víkingsson [b], Valérie Chosson [b], Asunción Borrell [a, *]

[a] Institute of Biodiversity Research (IRBio) and Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, Spain
[b] Marine and Freshwater Research Institute, Fornubuðir 5, 220, Hafnafjörður, Iceland

HIGHLIGHTS

- Stomach content samples from 25 whales from western Iceland were analysed.
- The only ingested prey found in the samples was northern krill.
- The average concentration in krill was 0.057 synthetic particles (SP) per gram.
- The number of SP daily ingested by fin whales was estimated in the tens of thousands.
- SP presence in their diet might facilitate the exposure of whale populations to POPs.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study we aim to assess the daily ingestion rates of synthetic particles by the fin whales (*Balaenoptera physalus*) that feed off the western coast of Iceland. To do so, we collected and analysed samples from the stomach content of 25 fin whales, consisting solely of northern krill (*Meganyctiphanes norvegica*). The particles found consisted of fibres and fragments, mainly blue, black and red, with an average size of 1.2 ± 1.3 mm. To confirm the synthetic nature of these particles, we used Micro-Fourier Transform Infrared Spectroscopy and comparison with a polymer library. The mean concentration of synthetic particles in the krill samples found in the stomachs of whales was 0.057 particles per gram, a value much lower than that previously reported for particle uptake by krill. From this concentration in krill, we estimated that the daily intake of synthetic particles for the North Atlantic fin whale would be ranging from 38,646 ± 43,392 to 77,292 ± 86,784 particles per day. Although at this level it is not possible to assess the impact of synthetic particles and their associated chemicals on the North Atlantic fin whale population, concentrations of these contaminants are likely to increase in the future, potentially causing adverse effects on whales and other marine mammals.

1. Introduction

Marine litter, broadly classified in macro-, meso-, micro- and nano-litter, encompass a group of manufactured or processed

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

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fragments and fibres of different shapes, textures and colours. It is composed of over 80% of plastic items. Every year, between 4.8 and 12.7 million tons of plastic reach the oceans (Jambeck et al., 2015), currently making up the most widespread and chronic type of marine pollution (Alimba and Faggio, 2019).

Plastics can act as vector for the transport of chemical compounds, which can be either directly related to plastic manufacture, providing it with certain plasticizing properties (*e.g.*, Phthalates such as mono and di-(2-ethylhexyl) phthalates (MEHP and DEHP, respectively) and Bisphenol A), or flame retardancy (*e.g.*, Polybrominated diphenyl ethers (PBDEs) or Organophosphate esters (OPEs)), or are pollutants that adsorb on the plastic, such as heavy metals and hydrophobic organic pollutants (*e.g.*, Polychlorinated biphenyls (PCBs), Chlorinated pesticides, Polycyclic aromatic hydrocarbons (PAHs)) (Avio et al., 2017). These pollutants, usually associated with plastic, may accumulate in the organisms and likely alter their biological processes. Among their multiple negative effects, they can act as endocrine disruptors, affecting organism reproduction and development (Mathieu-Denoncourt et al., 2015; Talsness et al., 2009), or depress the immune system, making it more vulnerable to viruses or other diseases (Aguilar and Borrell, 1994; Borrell et al., 1996).

Synthetic micro-litter is composed by microplastics, particles made of modified cellulose and cellulose combined with pigments (Lusher et al., 2020). Microplastics (*i.e.*, plastics items smaller than 5 mm) can be of primary origin (*i.e.*, beads, fibres or pellets) or of secondary origin (small plastic fragments derived from the breakdown of macroplastics) (Cole et al., 2013). Due to their small size, they are easily ingested by small aquatic organisms (*e.g.,* zooplankton) (Botterell et al., 2019; Khalid et al., 2020). This could represent a route to top predators through the food web (Nelms et al., 2018), although, to this date, studies certifying that this transfer involves biomagnification have not been produced (Provencher et al., 2019; Alava, 2020) and impacts from microplastics ingestion at high food web levels are not known (Reijnders et al., 2018).

Despite having been observed in laboratory within controlled feeding experiments (Cole et al., 2013), the ingestion of synthetic particles by euphausiids has not been fully confirmed in the field. Desforges et al. (2015) showed for the first time the ability of North Pacific krill (*Euphausia pacifica*) to ingest microplastics in the wild, suggesting that these animals may confuse microplastics with natural prey items when they are within the same size range.

Euphausiids make up the largest proportion of the diet of most baleen whales (Hewitt and Lipsky, 2018), which need to filter thousands of cubic meters of water every day to capture their food. During this activity, whales may ingest synthetic particles directly from the water (Fossi et al., 2012, 2014), or indirectly from their prey, if they are already contaminated with synthetic particles (Besseling et al., 2015; Germanov et al., 2018).

The fin whale (*Balaenoptera physalus*) is a cosmopolitan mysticete that carries out annual migrations from low-latitude breeding areas in winter to high-latitude feeding areas in summer (Aguilar and García-Vernet, 2018). The waters off western Iceland are a summer feeding ground for the North Atlantic fin whale population, which in this area feeds predominantly on the euphausiid *Meganyctiphanes norvegica* (Vikingsson, 1997). In this study, we investigated the presence of synthetic particles in the stomach of fin whales that feed off western Iceland. To do so, we analysed the krill obtained directly from the whales digestive tract, and basing on the results obtained, we assessed the magnitude of synthetic particle ingestion in this fin whale population.

2. Materials and methods

2.1. Sampling

Number, size, shape, type, and colour of synthetic particles were determined on different sets of samples of krill extracted from the forestomach (first compartment) of 25 fin whales and preserved frozen until analysis (Table 1). The whales sampled were caught during commercial whaling operations in the waters off western Iceland and flensed at the factory Hvalur H/F, located in Hvalfjörður, during summer 2018 (Fig. 1). Stainless steel material was used to cut through the stomach walls and manipulate the stomach contents, of which about 20 g per sampled whale were collected and placed in glass bottles. The krill extracted from the stomachs was carefully inspected in situ and no synthetic particles were observed in its jaw or exoskeleton. However, it was not rinsed with distilled water because we were interested in collecting all the synthetic particles from the samples. No field blanks were made, as weather and factory conditions did not facilitate this. The samples were stored at -20 °C, until their analysis in the laboratory.

2.2. Analysis of synthetic particles

Subsamples of krill of approximately 11 g (corresponding to ca 50 euphasiid individuals) were taken to guarantee a similar weight between samples and that sample was enough to perform other analyses (isotope and alkenone analysis). Samples were defrosted and placed into a glass beaker in 1:20 (w/v) H_2O_2 (15% H_2O_2, Chem-Lab, Germany) and heated at 55–65 °C until H_2O_2 evaporation. Aliquots of 10 ml H_2O_2 were added gradually to the beakers until all the organic matter was digested (Tsangaris et al., 2020). Samples were then diluted with 50 ml Milli-Q and vacuum-filtered on fibreglass filters (pore size 1.2 µm, Whatman, GE Healthcare, UK), which were dried at room temperature for 24 h and subsequently stored in Petri dishes. For more details, consult Garcia-Garin et al. (2019) and Tsangaris et al. (2020). Tsangaris et al. (2021) performed a harmonization exercise on the two principal methods of microplastic extraction from biological samples (*i.e.*, 15% H_2O_2 vs 10% KOH digestion), and microplastic recovery rates for the two methods were similar for each sample tested, with a mean recovery rate of 88.75% when using H_2O_2.

Filters were examined under a Nikon SMZ1000 stereomicroscope (10x to 40x) coupled with a DS-Fi2 camera. Synthetic particles found in the filters were photographed, counted, and classified by size (0.1–0.5, 0.5–1.0 and 1–5 mm), colour (blue, red, black and white) and shape (fragment, fibre and bead) (Lusher et al., 2020). Nineteen potential synthetic particles were analysed with a Thermo Scientific Nicolet™ iN™ MX µFT-IR (Micro-Fourier Transform Infrared Spectroscopy) microscopy, and then compared against a polymer library to identify the type of polymer, at the *Centres Científics i Tecnològics* of the University of Barcelona (CCiT-UB).

2.3. Quality assurance and quality control

To prevent contamination, researchers wore cotton lab coats and gloves. Air currents were reduced to a minimum throughout the analysis. The work was done in clean laboratory conditions. Glass or metal equipment was used instead of plastic (both in the field and in the laboratory). Glass beakers were rinsed with purified water and then dried before using. Krill samples were covered with aluminium foil during H_2O_2 digestion. A vertical laminar flow cabinet was used for sample filtration. Filters were protected with glass lids during stereoscope observation (Correia Prata et al., 2019). Procedural blanks, negative controls composed of 50 ml of 15%

Table 1
Biological parameters of the whales sampled, and synthetic particles (SP) found in their stomach content (*i.e.*, krill).

Whale code	Catch day	Sex	Body length (m)	Weight of sample analized (g)	Number of SP per sample	Number of SP per kg of sample
F18004	jun-18	Female	16.2	12.2	1	82
F18008	jun-18	Female	18.3	10.9	1	92
F18009	jun-18	Male	18.6	11.9	0	0
F18012	jul-18	Male	18.9	12.9	1	78
F18016	jul-18	Female	19.8	11.3	0	0
F18017	jul-18	Female	18.9	11.4	0	0
F18019	jul-18	Female	18.6	11.4	1	88
F18020	jul-18	Male	19.8	11.9	1	84
F18030	jul-18	Female	19.2	10.3	2	194
F18036	jul-18	Male	18.3	11.7	0	0
F18047	jul-18	Female	18.0	11.3	0	0
F18048	jul-18	Female	18.9	11.4	0	0
F18052	jul-18	Male	16.8	11.2	1	89
F18060	jul-18	Male	18.3	11.0	0	0
F18071	ago-18	Female	19.5	12.3	0	0
F18073	ago-18	Female	19.2	11.2	2	179
F18075	ago-18	Male	17.7	11.5	0	0
F18083	ago-18	Male	18.9	12.9	1	78
F18086	ago-18	Male	18.3	11.0	2	182
F18092	ago-18	Male	18.3	10.5	1	95
F18098	ago-18	Male	18.3	12.9	0	0
F18099	ago-18	Female	20.4	11.3	1	88
F18110	ago-18	Female	18.3	13.3	0	0
F18111	ago-18	Male	14.3	10.6	1	94
F18114	ago-18	Female	19.5	12.6	0	0
mean				**11.64**	**0.64**	**57**
SD				**0.81**	**0.70**	**64**

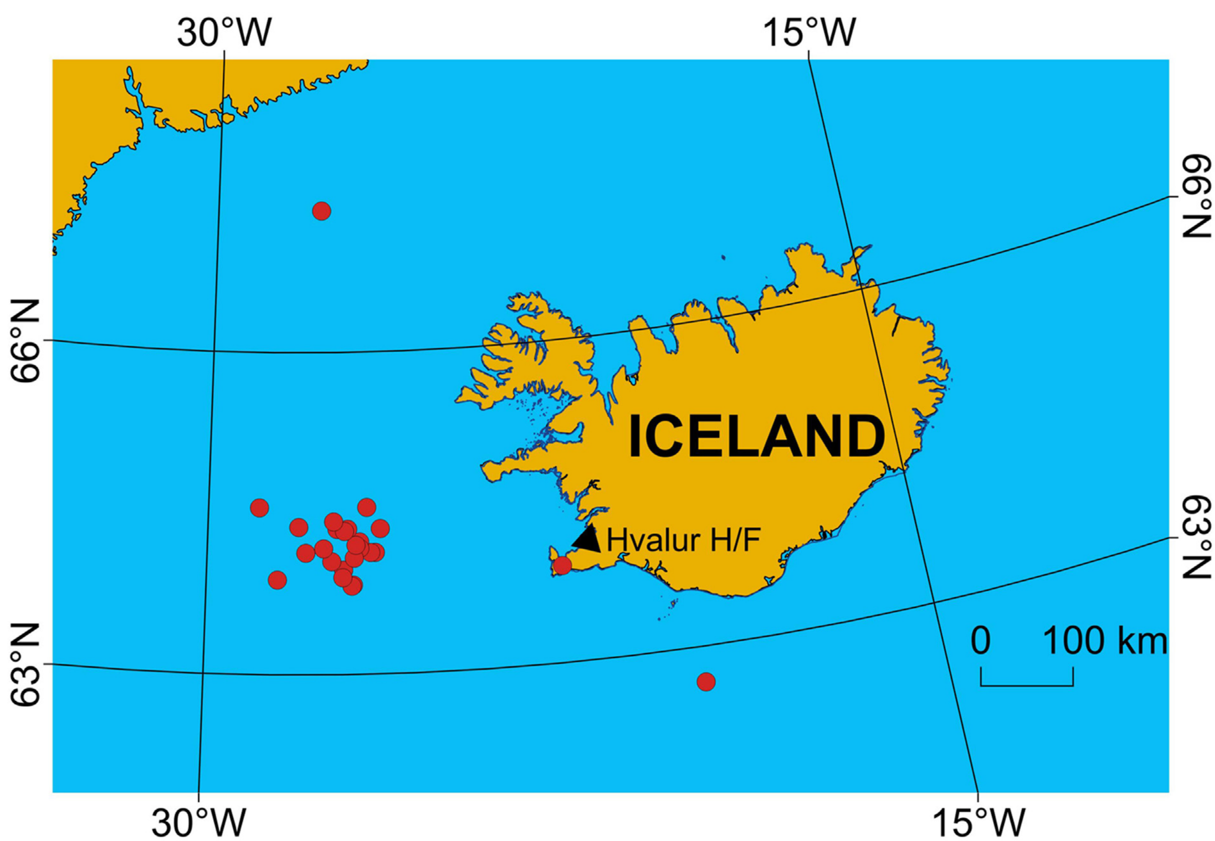

Fig. 1. Locations of fin whales catches (red dots) and of the whaling factory where whales were flensed (black triangle).

H_2O_2 (1 blank every 5 samples), or open petri dishes with a fibreglass filter (1 blank every 5 samples), were examined along with the samples (Correia Prata et al., 2019).

2.4. Quantification of synthetic particles ingested by fin whales

To quantify the synthetic particles ingested by fin whales, we based our calculations on the daily feeding rates estimated for the North Atlantic fin whale population by Víkingsson (1997), which ranged between 678 and 1,356 kg of krill, depending on the food transit time through the digestive system. We considered these values as the minimum and maximum amounts of krill ingested per day. To estimate the daily number of synthetic particles ingested by the whales, we multiplied these values for the number of synthetic particles detected in the krill samples (*i.e.*, 57 items/kg, Table 1).

3. Results

In total 19 particles were found in the 25 samples examined. One of them was excluded from the results as it was considered airborne contamination due to its similarity to one red fibre found in the blanks (Fig. S1). Out of the remaining 18 particles, one was a non-modified cellulose, one a silicate mineral, and the remaining 16 were considered synthetic particles (Fig. 2 and Fig. S2). Out of the 16 synthetic particles, five (37.5%) were identified as modified cellulose (*i.e.*, cellulose with pigments or rayon); three (18.8%) as polyethylene, three (18.8%) as polystyrene, three (18.8%) as polypropylene and one (6.1%) as acrylonitrile (Fig. S2).

The number of synthetic particles ranged from 0 to 2 per sample (Table 1). The frequency of occurrence, calculated as the percentage of samples with synthetic particles from the total number of samples, was 52%. The average concentration of synthetic particles per sample, considering all samples, was 0.64 ± 0.70 (mean ± SD), and that of synthetic particles per gram of krill was 0.057 ± 0.064 (Table 2).

The shape, colour and size of the synthetic particles extracted from the samples of stomach content are depicted in Fig. 3. The most frequent shape, colour and size of synthetic particles were fibres (69%), blue (62.5%) and the size smaller than 0.5 mm (44%), respectively.

The daily number of synthetic particles ingested by the whales was estimated to be between 38,646 and 77,292 (Table 2).

4. Discussion

This study investigates the ingestion rate of synthetic particles through the stomach contents of fin whales that feed in the waters off western Iceland during summer. The prey found in the stomach content of the sampled whales consisted exclusively of krill, consistently with a previous study on whales captured from the same population between 1967 and 1989. Of the 1,609 stomachs analysed in that study, 96% contained only euphausiids, 99% of which belonged to the species *M. norvegica* (Sigurjónsson and Víkingsson, 1997).

4.1. Synthetic particle abundance in krill

M. norvegica feeds by filtering from the water dense patches of prey organisms, especially copepods, while moving through the water column (Mauchline, 1967; McClatchie, 1985). This type of feeding could facilitate the ingestion of synthetic particles similar in size to their prey (Cole et al., 2013). The ingestion of synthetic particles by marine zooplankton is well documented through laboratory experiments (Cole et al., 2013), and it has been suggested to be a significant pathway of plastics into marine food webs (Set·ä·l·ä·et al., 2014). However, the levels of synthetic particles in zooplankton within natural marine environments remain largely unknown (Botterell et al., 2019).

The current study reports for the first time the presence of synthetic particles in *M. norvegica* in field samples. To our knowledge, only two studies assessed microplastic ingestion in other euphausiids species under natural conditions (Desforges et al., 2015; Sun et al., 2018). Desforges et al. (2015) analysed the ingestion of microplastics in North Pacific krill (*E. pacifica*) from the northeast Pacific Ocean, finding 0.058 microplastics/individual, and 0.83 microplastics/g. The large difference between this concentration and that of 0.057 synthetic particles/g we found in *M. norvegica* may be due to several factors. On the one hand, since the concentration of microplastics in the zooplankton correlates with that of seawater (Desforges et al., 2015), it could reflect different levels of synthetic particles and/or microplastic concentration in seawater, that is, higher concentrations in the Pacific than in the Atlantic Ocean (Van Sebille et al., 2015). On the other hand, it could reflect a greater capability of *E. pacifica*, for the acquisition and accumulation of synthetic particles, similarly to the capacity that this euphausiid has with respect to copepods (Desforges et al., 2015). More likely, it could be a combination of both factors.

Shape, colour and size of synthetic particles ingested by organisms should also vary, reflecting those of the particles in seawater. However, the types of particles found in the North Pacific krill (68% fibres, 32% fragments) was very similar to that of the synthetic particles found in the North Atlantic krill (69% fibres, 31% fragments), as well as their colour, mainly blue, black, and red (Desforges et al., 2015). On the other hand, the microplastics found in *E. pacifica* were considerably smaller (816 ± 108 μm) than those found in *M. norvegica* ($1,148 \pm 1,334$ μm). This could derive from differences in the size of the litter in the respective marine waters (Desforges et al., 2015), as well as from morphological characteristics of the two species, such as the length of the feeding appendix and the size of the mouth. Both sizes are larger in *M. norvegica* than in *E. pacifica* (Hewitt and Lipsky, 2018), which would allow the former to ingest relatively larger particles (Frost et al., 1983).

The other study reporting microplastic ingestion in krill under natural conditions analysed 10 zooplankton groups from the China Sea, including Euphausiidae spp. (Sun et al., 2018). The concentration of microplastics found in the krill was 0.2 items/krill (53% fibres), a much higher figure than the 0.058 reported by Desforges et al. (2015) and the 0.013 we found in the present study, probably reflecting the high level of contamination by plastics in the China Sea, up to 19.7 ± 22.4 microplastics/m^3 (Sun et al., 2018). Sun et al. (2018) did not specify differences between krill species, making any comparison between species unfeasible.

4.2. Number of synthetic particles ingested daily by whales

Fin whales are characterized by their extreme lunge-feeding behaviour that involves the engulfment of a large volume of prey-laden water (Goldbogen et al., 2007). To feed, the whale opens its mouth widely and collects dense shoals of prey (such as krill), along with large volumes of water. Then, it partially closes its mouth and presses its tongue against the upper jaw, forcing the water to pass sideways through the baleen, sieving out the prey, which are then swallowed. Since from the krill analyses we cannot distinguish the particles ingested by the krill from those attached to it from the surrounding water, the current estimate might include also part of plastics filtered directly from the water and retained with the prey.

A fin whale stomach can contain up to 600 Kg of krill (V·í·kingsson, 1997), making the analysis of synthetic particles

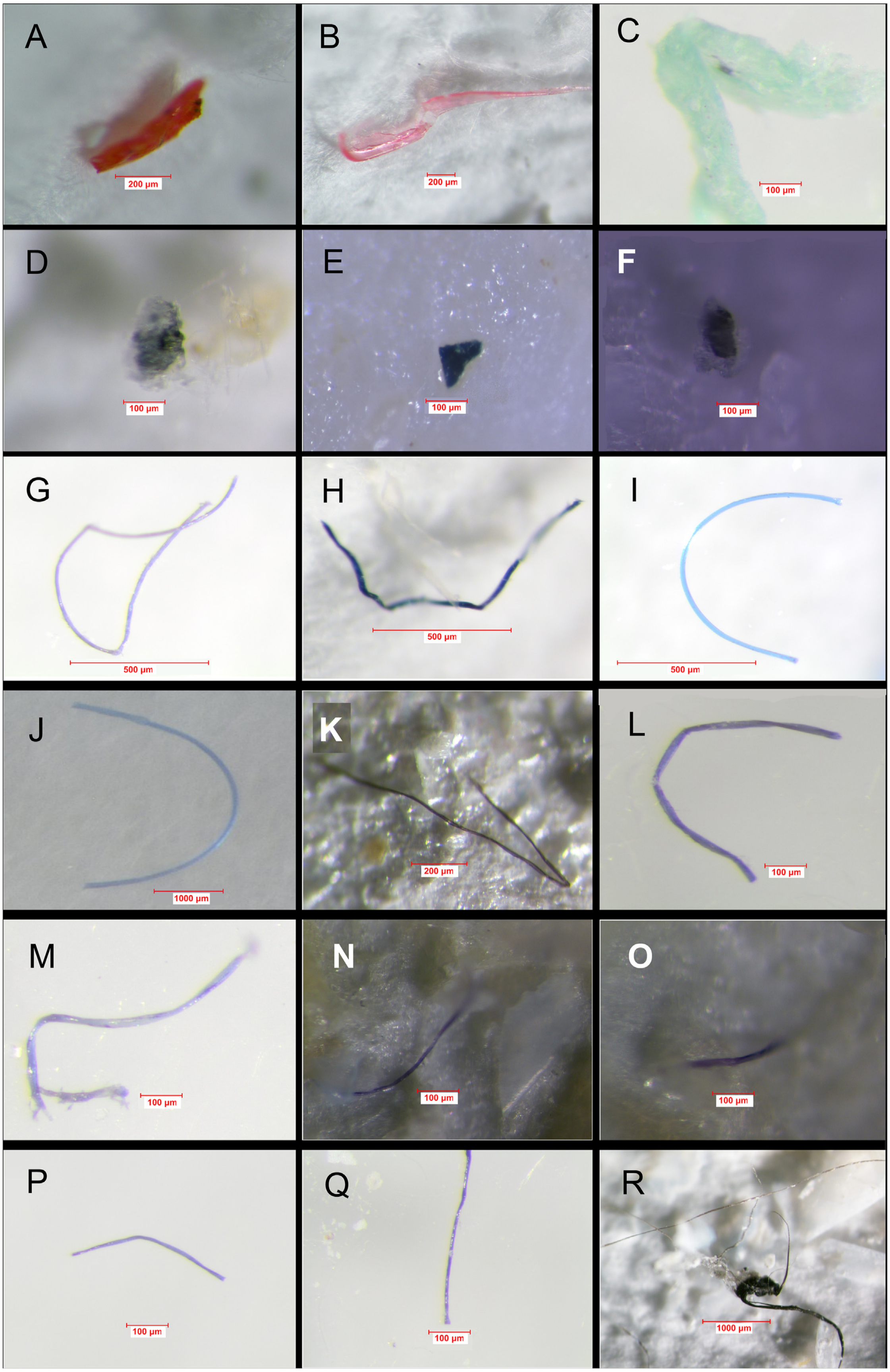

Fig. 2. Particles found in the 25 fin whales' stomachs. μFT-IR analysis revealed that the particles were composed of modified cellulose (H, L, M, N, O, P), polyethylene (A, Q, R), polystyrene (B, E, F), polypropylene (C, I, J), acrylonitrile (G), silicate mineral (D) and non-modified cellulose (K) (Fig. S2).

Table 2
Frequency of occurrence, characteristics and abundance of synthetic particles (SP) in the krill samples of stomach contents extracted from 25 fin whales from SW Iceland and estimation of total number of SP ingested daily by fin whales.

Parameter	Value
Number of samples containing SP	13
SP frequency of occurrence (%)	52
SP number	16
SP dimension length range (mm)	0.1–4.9
SP mean length (mm) (±SD)	1.2 ± 1.3
SP abundance in krill (mean ± SD):	
Number of SP per sample in all samples examined	0.64 ± 0.70
Number of SP per gram in all samples examined	0.057 ± 0.064
Number of SP per individual of krill (50 individuals/sample)	~0.0128
Calculation of synthetic particles ingested:	
Minimum-maximum kgs of krill ingested daily (min.-max.)	678-1,356
Number of SP per kg of krill (mean ± SD)	57 ± 64
Number of SP ingested daily (min. ± SD)	**38,646** ± **43,392**
Number of SP ingested daily (max. ± SD)	**77,292** ± **86,784**

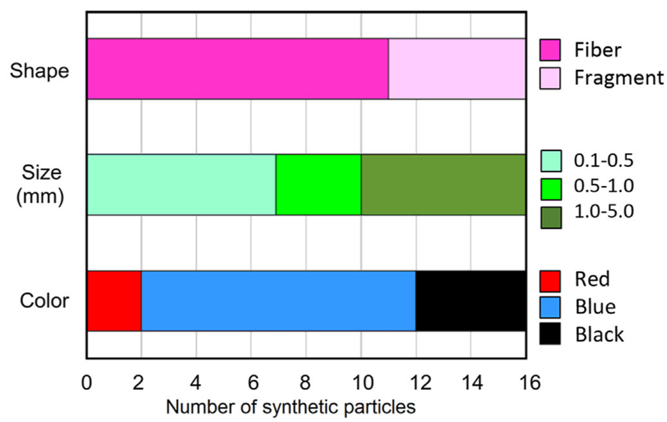

Fig. 3. Shape, size and colour of the synthetic particles detected in all the krill samples examined.

contained in the whole stomach content of 25 whales impossible to perform. For this reason, 11-g aliquots of the stomach content of each whale, equivalent to approximately 50 krill individuals, were analysed, and results were extrapolated to the total amount of krill ingested per day. Such small samples of krill per whale resulted in only 16 synthetic particles, which reduces the strength of the results obtained. Although the calculations may be poorly adjusted due to the factors discussed above, the extrapolation from the number of synthetic particles detected in the krill samples to the potential particles ingested daily by the whales results in an amount of several tens of thousands of particles per day.

Few studies have approached synthetic particle ingestion by whales, due to the difficulties involved. Fossi et al. (2014) calculated the potential amount of microplastics ingested by Mediterranean fin whales from the concentration of microplastics in the water where they were feeding, obtaining an average of 3,653 microplastics/day. However, they did not assess the microplastics ingested by the krill that the whales feed on, which probably produced a strong bias in their calculation.

Similarly to our approach, Desforges et al. (2015) calculated the ingestion of microplastics by humpback whales (*Megaptera novaeangliae*) off the coast of British Columbia, basing their estimations on the potentially ingested krill (*E. pacifica*). Since the krill was collected from seawater and not from the whales' stomach, their approach was made indirectly. Furthermore, the authors did not consider that these whales are generalist predators and that they likely exploit fish species in addition to zooplankton (Witteveen et al., 2011). The authors estimated a much higher intake of microplastics (above 300,000 items/day) than that of fin whales, despite the daily intake of krill by humpback whales is lower, probably because north pacific krill contained a larger amount of plastics than northern krill, as already discussed above.

On the other hand, Besseling et al. (2015) analysed the stomach content of a stranded humpback whale in the Netherlands. They found a total of 16 microplastics in samples from a gastrointestinal tract that represented only 5–10% of the intestine total length, which lead them to estimate a total of 160 microplastics in the whole intestine. This low number could be partly related to the fact that, since the whale spent four days agonizing stranded on a sandbank without ingesting anything, few remains of fish remained in its digestive tract. Moreover, the authors did not consider synthetic fibers in their analysis, which can also be a cause of the low estimate of microplastics in the gastrointestinal tract of the stranded whale.

Finally, Burkhardt-Holm and N'Guyen (2019) evaluated the possible uptake of microplastics by the common minke whale (*Balaenoptera acutorostrata*) and the sei whale (*Balaenoptera borealis*) based on the load of microplastics of their prey, but they did not quantify the number of microplastics ingested.

Apart from the above cited, we are not aware of any other research estimating the amount of synthetic particles ingested by mysticetes. While the effects of macro-litter ingestion in cetaceans are well known (*e.g.* Baulch and Perry, 2014), micro-litter ingestion in these species, especially in mysticetes, remains poorly studied (due to difficulty in sampling and analysing and lack of standardization, among others) (Zantis et al., 2021). Although most of the ingested particles are excreted in the faeces, their rates of ingestion and excretion are unknown. The likely disintegration of these particles, and the release and subsequent absorption of lipophilic contaminants through the gastrointestinal wall of the animal almost certainly depends on the transit time in the digestive system. Advancing these types of studies, and harmonizing the quantification systems to allow more accurate intra- and inter-specific comparisons among baleen whales, should be a priority for a future in which the quantity of synthetic particles will exponentially increase in the marine environment.

4.3. Review of the chemical compounds found in Icelandic fin whales related to synthetic particles

Plastics can pollute the environment or the organisms that ingest them by releasing several additives and chemical compounds that are attached to them. Contaminants associated with marine litter include chemical additives, such as plasticizers, antioxidants, flame-retardants and UV-stabilizers, and chemicals that accumulate from the surrounding ocean waters (Avio et al., 2017; Rochman, 2015). Most of these compounds are highly recalcitrant, such as the so called 'persistent organic pollutants' (POP), meaning that their chronic acquisition produces an accumulation over time along the trophic webs and ends up depressing the immune system and acting as endocrine disruptor in terminal predators. Ingestion of these compounds usually occurs through food, but since they are bound to plastics, a high exposure to microplastics can lead to an increase in the body loads of these pollutants (Hermabessiere et al., 2017).

Many of these pollutants have previously been detected in the tissues of the Icelandic fin whale population (Borrell, 1993; Garcia-Garin et al., 2020; Rotander et al., 2012). Thus, Garcia-Garin et al. (2020) recently found organophosphate esters in samples of muscle of fin whales and of krill from Icelandic waters at concentrations of 1,060 (SD = 2,564) and 949 (SD = 1,090) ng/g lw, respectively.

Furthermore, Borrell (1993) had previously found organochlorine compounds (PCBs and DDTs) in concentrations of few μg/g lw, in the blubber of individuals caught in 1986 from the same population. Finally, Rotander et al. (2012) found organobrominated compounds (PBDEs) in the blubber of individuals sampled during the 1980s and 2006–2009 in Iceland. The highest levels of these compounds were found in the most recent samples (8.4, 1980s vs 22 ng/g lw, 2006–9), which possibly reflected the increase of the global production of technical PBDE mixtures during those years. Given that in 2009 the Parties of the Stockholm Convention for POPs included the commercial PBDEs in the list of prohibited substances, it would be interesting to investigate the current progression of these pollutants in the Icelandic fin whale population.

Some of the aforementioned pollutants found in the whales' tissues could derive from the ingestion of plastic particles throughout the life of the animals. No direct effects of these toxic compounds have been described in this species yet, but other marine mammals feeding on higher trophic level prey (*e.g.*, dolphins and seals) tend to accumulate higher amounts of POPs and have shown reproductive and immunosuppressive effects (*e.g.*, Reijnders, 1986; Aguilar and Borrell, 1994; Jepson et al., 2016). However, the effects of synthetic particles in the natural environment and implications for the food web remain poorly understood (Hermsen·et al., 2018). Further studies are needed to evaluate the possible toxic effects caused by the ingestion of synthetic particles and their adhered pollutants by mysticete whales.

5. Conclusions

A total of 57 synthetic particles per kg krill were found in the stomach content samples of fin whales from the waters off western Iceland, which, according to our estimations, would imply that an individual in this population could ingest between 38,646 and 77,292 synthetic particles per day. Despite no toxic effects have been reported for these organisms as caused by the ingestion of micro particles, this amount of litter seems high enough to fear that pollutants associated with synthetic marine litter could be transferred to fin whale tissues, potentially causing adverse effects, in an uncertain future.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.130564.

Credit author statement

Odei Garcia-Garin: Conceptualization, Formal analysis, Methodology, Investigation, Writing - review & editing. **Alex Aguilar:** Funding acquisition, Writing - review & editing. **Morgana Vighi:** Writing - review & editing. **Gísli A. Víkingsson:** Providing samples, Writing - review & editing. **Valerie Chosson:** Providing samples, Writing - review & editing. **Asunción Borrell:** Conceptualization, Funding acquisition, Writing–Original Draft, Writing - review & editing.

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2.3. No evidence of microplastics in Antarctic fur seal scats from a hotspot of human activity in Western Antarctica

Autores: Odei Garcia-Garin[1], Iván García-Cuevas[1], Massimiliano Drago[1], Diego Rita[1], Mariluz Parga[2], Manel Gazo[1,2], Luis Cardona[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain
[2]SUBMON - Marine Environmental Services, Ortigosa 14, 08003 Barcelona, Spain

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Abstract:
Microplastics are present in almost all seas and oceans, including the Southern Ocean. To the south of the Antarctic Polar Front, microplastics are present mainly west to the Antarctic Peninsula, but information is scarce about their impact on the pelagic food web. Here, we analysed 42 scats of male Antarctic fur seals (*Arctocephalus gazella*) collected in late summer at Deception Island (South Shetland Islands), which allowed us to assess the presence of microplastics in the pelagic food web of the Bransfield Strait (Western Antarctica). Furthermore, we analysed the hard remains of prey in the scats to characterize the diet of fur seals. Hard remains recovered from the scats revealed that male Antarctic fur seals foraged on krill and myctophid fishes during late summer. Fourier-transform infrared spectroscopy (FT-IR) revealed that none of the seven fragments and three fibres recovered from their scats were microplastics, but rather were silicate minerals and chitin. These results suggest that the levels of microplastic pollution in the pelagic food web of the Bransfield Strait are extremely low.

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Short Communication

No evidence of microplastics in Antarctic fur seal scats from a hotspot of human activity in Western Antarctica

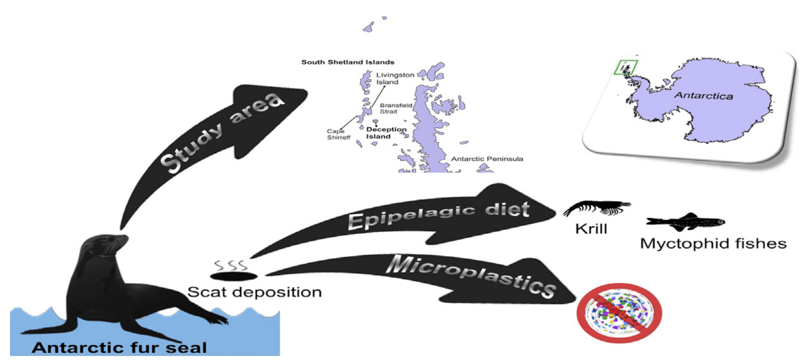
Odei Garcia-Garin [a,*], Iván García-Cuevas [a], Massimiliano Drago [a], Diego Rita [a], Mariluz Parga [b], Manel Gazo [a,b], Luis Cardona [a]

[a] Department of Evolutionary Biology, Ecology and Environmental Sciences and Biodiversity Research Institute (IRBio), Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain
[b] SUBMON - Marine Environmental Services, Ortigosa 14, 08003 Barcelona, Spain

HIGHLIGHTS

- Male Antarctic fur seals hauling out at Deception Island during late summer consume krill and mesopelagic fishes.
- Male fur seal scats were sieved and partially digested by KOH 20% to detect microplastics.
- Microplastics were absent from the scats of Antarctic fur seals.
- If present, microplastics occur at very low levels in the pelagic food web of the Bransfield Strait.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics are present in almost all seas and oceans, including the Southern Ocean. To the south of the Antarctic Polar Front, microplastics are present mainly west to the Antarctic Peninsula, but information is scarce about their impact on the pelagic food web. Here, we analysed 42 scats of male Antarctic fur seals (*Arctocephalus gazella*) collected in late summer at Deception Island (South Shetland Islands), which allowed us to assess the presence of microplastics in the pelagic food web of the Bransfield Strait (Western Antarctica). Furthermore, we analysed the hard remains of prey in the scats to characterize the diet of fur seals. Hard remains recovered from the scats revealed that male Antarctic fur seals foraged on krill and myctophid fishes during late summer. Fourier-transform infrared spectroscopy (FT-IR) revealed that none of the seven fragments and three fibres recovered from their scats were microplastics, but rather were silicate minerals and chitin. These results suggest that the levels of microplastic pollution in the pelagic food web of the Bransfield Strait are extremely low.

1. Introduction

The presence of plastic debris, including fragments smaller than 5 mm in size (hereafter microplastics, according to Arthur et al., 2009), has been reported from almost all seas and oceans of the world, from the Arctic (*e.g.*, Kanhai et al., 2018; Kühn et al., 2018) to

* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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the Southern Ocean (*e.g.*, Bessa et al., 2019; Jones-Williams et al., 2020; Lacerda et al., 2019; Le Guen et al., 2020; Suaria et al., 2020; Waller et al., 2017). The number of microplastic particles in a specific area depends on several factors, but the highest density has been reported from areas within or near the major ocean gyres (*e.g.*, Cózar et al., 2014; Law et al., 2010; Lebreton et al., 2018; Ryan, 2014) and in proximity to densely populated urban centres, touristic areas and shipping routes (Garcia-Garin et al., 2019, 2020b; Suaria and Aliani, 2014).

Antarctica is considered the most pristine environment on the planet, due to a very small and mostly seasonal human presence (Waller et al., 2017). Furthermore, the Antarctic surface waters are isolated from the rest of the world's oceans by a convergence zone (*i.e.*, the Antarctic Polar Front), which is the transition between Antarctic and subantarctic surface waters (Chown et al., 2015; Foster, 1984). Thus, it is hardly surprising that microplastics > 300 μm were not detected in most surface waters off Antarctica (Isobe et al., 2017; Kuklinski et al., 2019; Suaria et al., 2020; Waller et al., 2017), except those close to the South Shetland Islands and the Western Antarctic Peninsula (Jones-Williams et al., 2020). This is the Antarctic region with the highest human presence (Hughes and Ashton, 2017; Waller et al., 2017) and, hence, is the most susceptible to local microplastic pollution. Microplastics also occur in coastal sediments close to research stations (Munari et al., 2017; Reed et al., 2018; Sfriso et al., 2020; Waller et al., 2017).

Apex predators with a broad diet have the potential for integrating microplastics from a diversity of species, particularly those predators that consume their prey whole (Fossi et al., 2018; Perez-Venegas et al., 2018, 2020; Wright et al., 2013). The burden of microplastics can be detected in low-density areas by focusing on predators such as birds (*e.g.*, Ryan et al., 2016); penguins (*e.g.*, Bessa et al., 2019), cetaceans (*e.g.*, Besseling et al., 2015; Garcia-Garin et al., 2020a) and pinnipeds (*e.g.*, Nelms et al., 2019a; Perez-Venegas et al., 2020). Indeed, microplastics have been found in the scats of Antarctic apex predators, such as penguins (Bessa et al., 2019; Panasiuk et al., 2020).

Twelve out of thirty-two seal species have been recorded to ingest plastic at least occasionally (Kühn et al., 2015), and fur seals have proven to be a good indicator of microplastic pollution in the Southern Hemisphere (*e.g.*, Perez-Venegas et al., 2018, 2020). They usually feed on krill and mesopelagic fishes, which often ingest microplastics (Boerger et al., 2010; Desforges et al., 2015). Indeed, animals foraging on krill are more likely to accidentally ingest microplastics than other species foraging at higher trophic levels (Besseling et al., 2015). Fur seals as a group have been proposed to be bioindicators of microplastic pollution (Perez-Venegas et al., 2020) and, thus, the Antarctic fur seal *Arctocephalus gazella* in particular may be considered a potentially good bioindicator species for assessing the presence of microplastics in pelagic Antarctic ecosystems, as it has been reported to ingest microplastics north of the Antarctic Polar Front (Eriksson and Burton, 2003). The population of Antarctic fur seals breeding in the Western Antarctic Peninsula (Hofmeyr, 2016) is of interest, because this is the Antarctic region with the highest human presence (Hughes and Ashton, 2017; Waller et al., 2017) and, hence, is the most susceptible to local microplastic pollution (Jones-Williams et al., 2020).

The aim of this study was to determine the presence of microplastics in the coastal waters of the Bransfield Strait, specifically by analysing Antarctic fur seal scats collected at Deception Island during late summer. The scat analysis was performed by following the methodology used previously by Perez-Venegas et al. (2018, 2020) and Eriksson and Burton (2003). To determine potential indirect pathways of microplastic ingestion, we also identified the hard remains present in the scats to better characterize the diet of fur seals.

2. Materials and methods

2.1. Ethics statement

Field work was approved by the Spanish Polar Institute (permit #CPE-2018-4).

2.2. Study area and sampling

Fieldwork was conducted on Deception Island, which hosts one of the main haul-out sites of male Antarctic fur seals in the South Shetland Islands (Fig. 1). Males arrive at Deception Island in mid-February, once the breeding season has ended at the Cape Shirreff rookery (Livingston Island, South Shetland Islands). Fresh scats (n = 42) were collected using a small metallic shovel at the same beach on February 25th and March 10th, and they were then stored at −20 °C, wrapped in aluminium foil (Correia Prata et al., 2019). We did not use field blanks.

2.3. Diet characterization

Scats were defrosted at 5 °C and weighed. Scats were split in two subsamples for latter analysis. A subsample (10–12 g) was collected from each scat and preserved for microplastic analysis. The remaining scat (35–380 g) was passed through sieves with mesh sizes of 3.0, 1.0 and 0.5 mm, and the hard remains of recovered prey were identified to the lowest possible taxonomic level by following Reid (1996) and the AFORO database of fish otoliths (Lombarte et al., 2006). Unidentified remains were not considered in any further analysis. We characterized

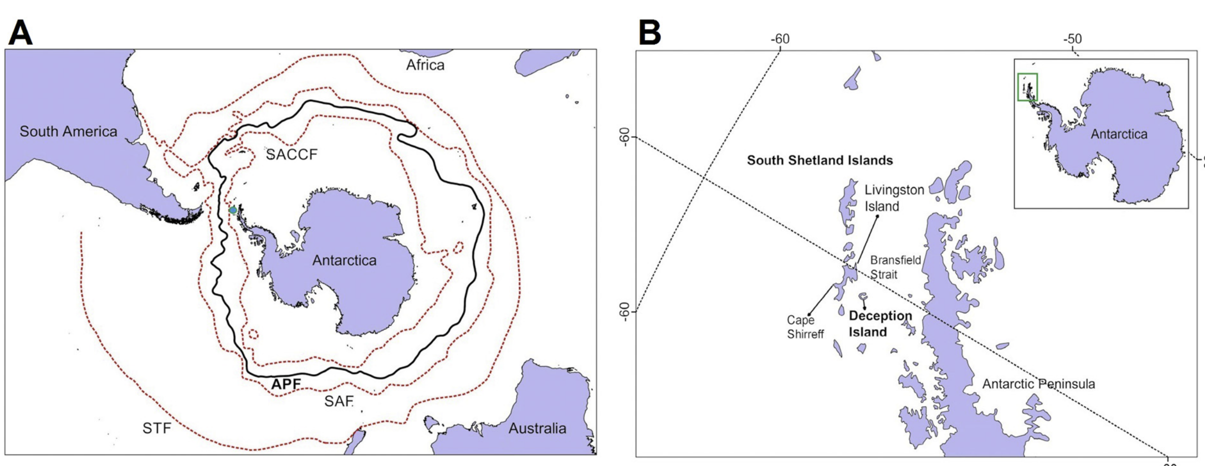

Fig. 1. A) Mean positions of the Southern Antarctic Circumpolar Current Front (SACCF), the Antarctic Polar Front (APF), the Subantarctic Front (SAF) and the Subtropical Front (STF). The current study area is depicted with a green circle. Mean positions were obtained from Orsi and Harris (2019). B) The Bransfield Strait and Deception Island (QGIS Development Team, 2018). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

each prey species' importance to the diet by frequency of occurrence, *i.e.*, the percentage of samples in which a taxon was observed.

2.4. Microplastic analysis

Microplastics were extracted from the scats using two different methodologies in order to allow comparison with previous studies.

To remove organic matter and enable the detection of microplastics, one subsample of 10–12 g (approximately a 6% mean of the scat) from the inner part of the scat was dissolved in KOH 20%, in accordance with Perez-Venegas et al. (2018) and Lusher and Hernandez-Milian (2018). Each subsample was placed into a glass beaker with 20 mL g^{-1} wet weight for seven days. Samples were then vacuum filtered through fibreglass filters (pore size 1.2 μm, Whatman, GE Healthcare, UK), then dried at room temperature for 24 h and subsequently stored in Petri dishes covered with aluminium foil. Filters were examined under a Nikon SMZ1000 stereomicroscope coupled with a DS-Fi2 camera and standard stainless-steel tweezers. We inspected Whattman filters carefully to avoid loss of any possible microplastic attached to the filter.

At the same time that the scats were broken apart with water to extract the hard parts of prey, they were also inspected to detect microplastics, in accordance with Eriksson and Burton (2003). The scats were passed individually through sieves using mesh sizes of 3.0, 1.0 and 0.5 mm. Once again, the mesh sizes were above 0.5 mm, all particles smaller than this were lost. Sieves were examined under an Olympus SZ30 binocular loupe.

A total of 11 potential microplastics were found and photographed. Fourier-transform infrared spectroscopy (FT-IR) was used to identify the polymer type of particles detected (Correia Prata et al., 2019). Although μFTIR is more reliable for small sizes, many peer-review studies investigating microplastics in the environment used FT-IR as a valid method (*e.g.*, Digka et al., 2018; Sbrana et al., 2020). Furthermore, fibres found were large enough to be checked with FT-IR. FT-IR analysis was carried out with a PerkinElmer Frontier FT-IR spectrometer at the Scientific and Technological Centres of the University of Barcelona (CCiTUB) using a self-generated polymer library (Digka et al., 2018; Garcia-Garin et al., 2019). The confidence level for comparing the sample spectrum to that of the self-generated library database was set to 60%.

To prevent contamination throughout the analysis, the researchers performing the analyses wore cotton lab coats (Correia Prata et al., 2019), and air currents were reduced to a minimum. All glass beakers were rinsed with purified water, and the scat samples were covered with aluminium foil during KOH digestion. The aluminium foil was replaced every day to avoid degradation and contamination of the samples. A vertical laminar flow cabinet was used for sample filtration. Procedural blanks (Correia Prata et al., 2019) were used during all steps (1 blank every 10 samples). Three blue fibres were found in one blank (Fig. 3A). We excluded one fibre from the results that was very similar to those found in the blanks, as it was considered airborne contamination.

3. Results

The hard remains recovered from the scats revealed a strictly pelagic diet dominated by Antarctic krill *Euphausia superba* and three myctophid fishes: *Gymnoscopelus nicholsi*, *Gymnoscopelus braueri* and *Electrona antarctica* (Fig. 2). The feathers of an unidentified penguin were observed in one scat.

Five possible microplastic fragments were discovered after sieving the scats, and an additional three fibres and two fragments of possible microplastics were discovered after dissolving and filtering a subsample from each scat (n = 42) (Fig. 3B & C). However, FT-IR analysis revealed that none of these particles were microplastics but were instead silicate minerals and chitin.

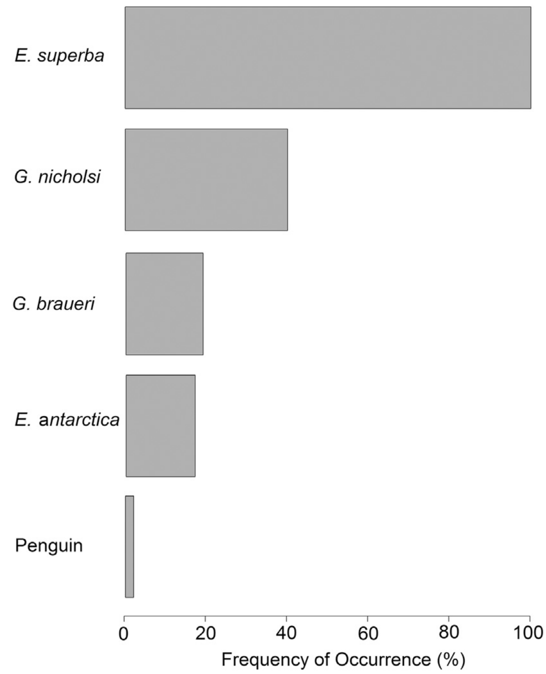

Fig. 2. Frequency of occurrence of prey species recovered from 42 male Antarctic fur seal scats at their Deception Island haul-out in late summer (February 22nd to March 29th).

4. Discussion

The results reported here reveal that male Antarctic fur seals hauling out at Deception Island during late summer foraged on krill and myctophid fishes and their scats were free from microplastics.

Satellite tracking of male Antarctic fur seals tagged at Collin Point has revealed that they remained within the Bransfield Strait in February and March 2019 (Cardona, unpublished results) and, hence, the scats collected for the present study likely represent foraging between the South Shetland Islands and the Antarctic Peninsula. This is extremely important, because this is the region with the highest concentration of human activity in Western Antarctica (Hughes and Ashton, 2017) and hence the most likely to experience microplastics pollution from local sources (Jones-Williams et al., 2020). It should be noted that females breeding at nearby Cape Shirreff, on Livingston Island, forage mainly west and north to the South Shetland Islands (Arthur et al., 2018) and hence, any eventual microplastic analysis conducted on scats from the Cape Shirreff rookery would be informative of a region with much lower direct human activity and microplastic burden.

As previously reported, male Antarctic fur seals hauling out at Deception Island foraged mainly on Antarctic krill and myctophid fish (Bonner, 1968; Davis et al., 2006; Goldsworthy et al., 1997). Gut transit time in pinnipeds is at most five days (Hall-Aspland et al., 2011), and most of the trips made by the male Antarctic fur seals hauling out at Deception Island lasted <48 h (Cardona, unpublished data). This suggests that the scats collected at Deception Island offer a good representation of the fur seal diet during late summer, as only a few scats could have been lost at sea during the longest trips. Despite the short gut transit time of pinnipeds (Hall-Aspland et al., 2011), the stomachs of marine mammals are considered to be sites where microplastics are temporarily retained (Nelms et al., 2019a), as far as they are present in the guts of their prey.

Suspension-feeding zooplankton have ingested microplastics in laboratory experiments (Cole et al., 2013), and euphausiids have been observed to ingest microplastics in field studies at levels of 1 particle per every 17 euphausiids (Desforges et al., 2015). Additionally, recent research has confirmed that microplastics, although at low concentrations, are being consumed by zooplankton in the Southern Ocean

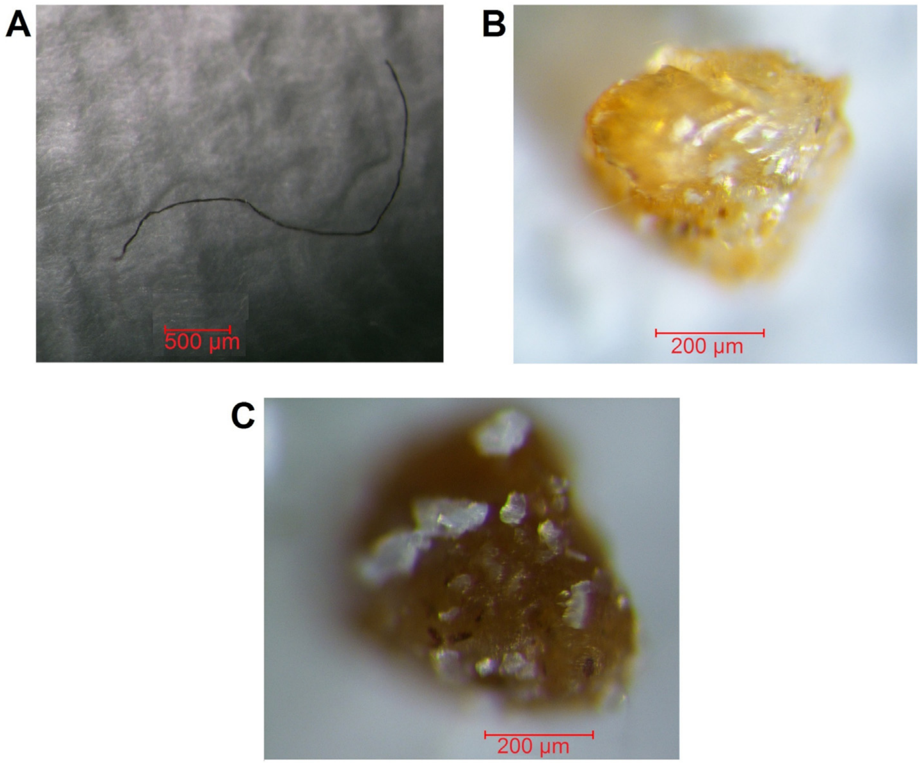

Fig. 3. Examples of a fibre found in a blank (A), and fragments (B and C) found in male Antarctic fur seal scats from Deception Island and discarded as microplastics by FT-IR analysis.

(Jones-Williams et al., 2020). Furthermore, mesopelagic fish, particularly myctophid fish, also ingest microplastics (Boerger et al., 2010; Bernal et al., 2019; Davison and Asch, 2011; van Noord, 2013). Thus, the presence of microplastics is expected in marine mammals when their prey, such as euphausiids and myctophid fish, ingest microplastics (Perez-Venegas et al., 2020). Based on this rationale, pinnipeds have been proposed as indicators of microplastic pollution (Perez-Venegas et al., 2020). Therefore, if microplastics existed in the pelagic food web of the Bransfield Strait, we expected to find them in the scats of Antarctic fur seals from Deception Island. However, microplastics were not detected in our samples.

Eriksson and Burton (2003) were the first to detect microplastics in the scats of fur seals in the Southern Hemisphere, with levels ranging from one to four microplastics per scat of the subantarctic fur seal *Arctocephalus tropicalis* and of Antarctic fur seals from Macquarie Island. Conversely, Ryan et al. (2016) did not find microplastics in the scats of fur seals from Macquarie Island, likely because of low levels of microplastic pollution in their nearby foraging grounds. Later, Perez-Venegas et al. (2018) reported microplastics from the scats of the South American fur seal *Arctocephalus australis* from Guafo Island (Northern Chilean Patagonia), with levels ranging from 0 to 180 units per scat. We used similar methods here, so methodological biases are unlikely to explain why we did not detect microplastics in the present study. Certainly, we did not conduct field blanks, so contamination from the soil could not be ruled out if we had found microplastics in the scats. This should be considered in future studies addressing other haul-out sites, but it does not diminish the value of the results reported here, as we did not find any microplastics.

The absence of microplastics in the scats of Antarctic fur seals from Deception Island also contrasts with the presence of microplastics in the scats of King penguins (*Aptenodytes patagonicus*) from South Georgia (Le Guen et al., 2020) and gentoo penguins (*Pygoscelis papua*) from South Georgia and the South Orkney Islands (Bessa et al., 2019). Those species forage in areas north to the Bransfield Strait, but they also differ from Antarctic fur seals in a high prevalence of fish in their diets (Berón et al., 2002; Cherel et al., 2002). Thus, the higher burden of microplastic pollution reported from the scats of penguins may result from differences in diets, foraging grounds and their capacity to retain the microplastics present in their prey. Indeed, microplastic levels can also be very low in pinnipeds inhabiting highly polluted regions (Nelms et al., 2019b), which suggests that further research is needed to better understand the potential role of pinnipeds as bioindicators of microplastic pollution and whether negative results such as in Bourdages et al. (2019) are indicative of microplastic absence in the environment.

In addition, it should be noted that one of this study's methods, which followed Eriksson and Burton (2003), allows detecting only microplastics larger than 0.5 mm. It is also worth noting that only 42 seal scats were analysed. Further research that directly looks for microplastics in the guts of krill, myctophid fishes, and dead stranded fur seals would provide further insights into the prevalence of microplastic pollution in the pelagic ecosystem of the Bransfield Strait and elsewhere off Antarctica. Another relevant point is the northward migration of female fur seals during winter (Arthur et al., 2018), which leads them to areas with higher levels of microplastic pollution (Perez-Venegas et al., 2018). Information are scarce about the winter habitat of male fur seals breeding in the South Shetland Islands, but they would presumably be less exposed than females to microplastic pollution if they remained south of the Antarctic Polar Front year-round. This merits further research.

In any case, this is the first study looking for microplastics in the scats of any fur seal species south of the Antarctic Polar Front, and the different findings of studies conducted north of this front (Eriksson and Burton, 2003; Perez-Venegas et al., 2018) suggest higher levels of microplastic pollution at lower latitudes. The results reported here fit the overall evidence indicating that the Antarctic waters, and particularly the pelagic compartment of the Bransfield Strait, are less polluted by microplastics than the surrounding oceans (Bessa et al., 2019; Kuklinski et al., 2019; Suaria et al., 2020; this study). Floating debris are accumulating in the South Atlantic gyre as far south as 34–35°S (Ryan, 2014), and considerable microplastic concentrations have been reported from the South Indian Ocean subtropical gyre (Bernal et al., 2019). Data about microplastic levels in subtropical and subantarctic surface waters are abundant (*e.g.*, Cózar et al., 2014; Suaria et al., 2020), but studies looking for microplastics in Antarctic surface water have reported either none (Kuklinski et al., 2019) or very low levels of microplastics (Bessa et al., 2019; Cincinelli et al., 2017; Cózar et al., 2014; Eriksen et al., 2014; Isobe et al., 2017; Lacerda et al., 2019; Suaria et al., 2020; Waller et al., 2017).

The low levels of microplastic pollution in Antarctic waters may be because the Antarctic Polar Front (Fig. 1A) effectively blocks the arrival of microplastics from more humanized areas (Chown et al., 2015; Foster, 1984). The Antarctic Polar Front results from strong circumpolar currents and winds that promote the eastward movement of surface water, but they impede any north–south exchange (Chown et al., 2015). However, Antarctica is not completely isolated because the Antarctic Polar Front meanders seasonally and, thus, eddy formation may result in the transferal of some material across the Antarctic Polar Front (Fraser et al., 2011, 2017; Waller et al., 2017). Furthermore, animals (*e.g.*, seabirds) that usually cross this front could carry plastics to the Antarctic region from the northern polluted areas.

In conclusion, these results reveal very low levels of microplastic pollution in one of the major pelagic predators inhabiting one of Antarctica's hotspots of human activity.

CRediT authorship contribution statement

Odei Garcia-Garin: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Iván García-Cuevas:** Methodology, Investigation. **Massimiliano Drago:** Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition. **Diego Rita:** Resources, Writing - original draft, Writing - review & editing. **Mariluz Parga:** Resources, Writing - original draft, Writing - review & editing. **Manel Gazo:** Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing. **Luis Cardona:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

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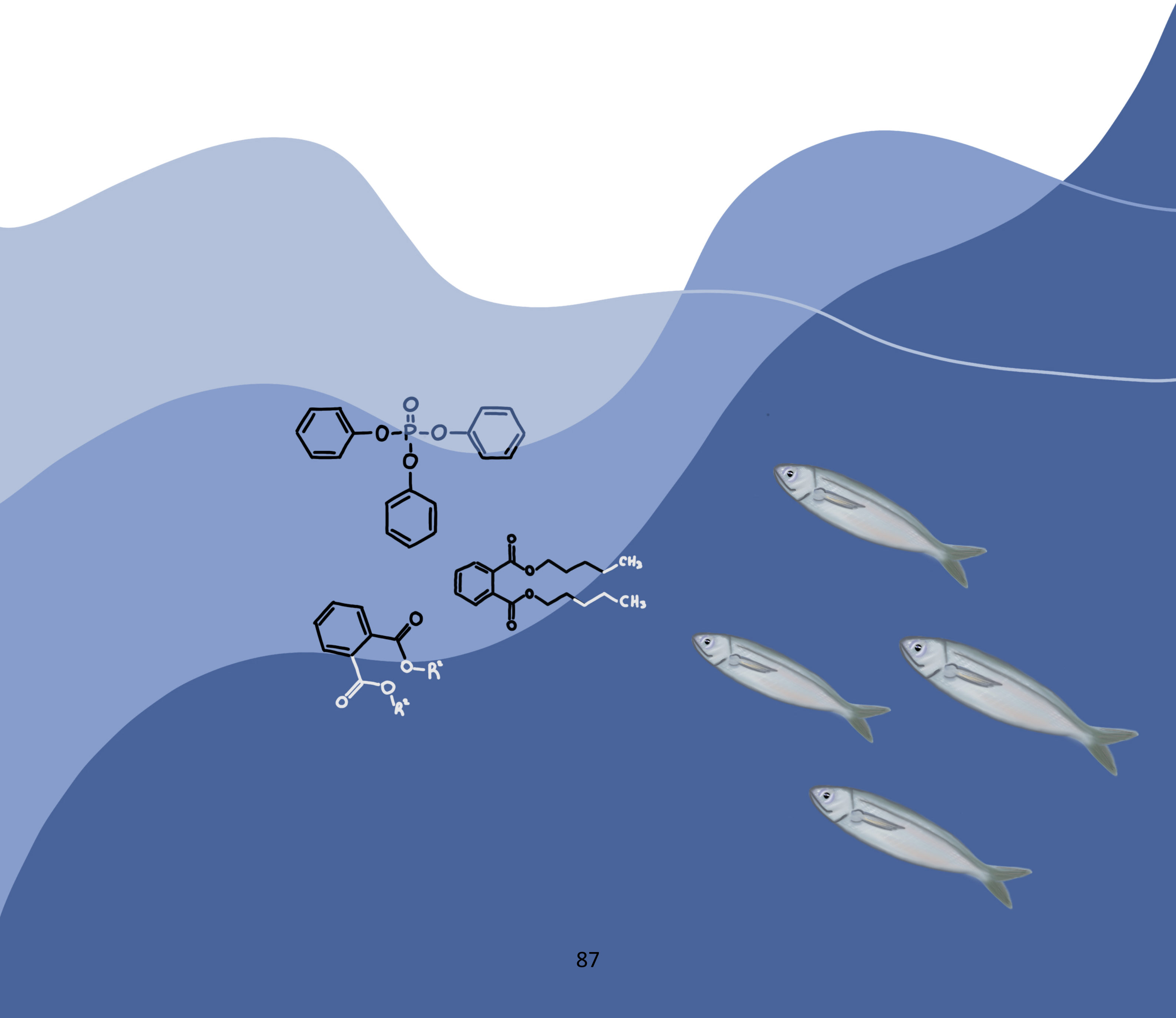
3. ADITIVOS PLÁSTICOS



3.1. Assessment of organophosphate flame retardants in Mediterranean *Boops boops* and their relationship to anthropization levels and microplastic ingestion

Autores: Odei Garcia-Garin[1], Morgana Vighi[1], Berta Sala[2], Alex Aguilar[1], Catherine Tsangaris[3], Nikoletta Digka[3], Helen Kaberi[3], Ethel Eljarrat[2], Asunción Borrell[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain
[2]Water, Environment and Food Chemistry, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEACSIC),
Jordi Girona 18-26, 08034, Barcelona, Spain
[3] Institute of Oceanography, Hellenic Centre for Marine Research, Athens, Greece

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Abstract:
Plastic litter pollution is increasing in the seas and oceans worldwide, raising concern on the potential effects of plasticizer additives on marine fauna. In this study, muscle samples of 30 bogues (*Boops boops*; Linneaus, 1758) from the North Western Mediterranean Sea were analysed to assess the concentrations of 19 organophosphate flame retardant (OPFR) compounds and to inspect any relationship with microplastic ingestion and relative levels of anthropization. Out of the 19 OPFRs analysed, 6 compounds were detected, being tri-n-butyl phosphate (TNBP), 2-ethylhexyldiphenyl phosphate (EHDPP) and triphenylphosphine oxide (TPPO) the most abundant. As expected, OPFR concentrations were higher in samples collected off the most anthropized area of the city of Barcelona than in those from the Cap de Creus Marine Protected Area, while no significant correlation was detected between OPFR concentrations and microplastic ingestion. The results of this manuscript provide a first evidence of OPFR presence in the muscle of the bogue and identify the coastal area off Barcelona as a possible concentration area for contaminants, further supporting the use of the bogue as an indicator species of plastic pollution in the Mediterranean Sea.

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Assessment of organophosphate flame retardants in Mediterranean *Boops boops* and their relationship to anthropization levels and microplastic ingestion

Odei Garcia-Garin [a,*], Morgana Vighi [a], Berta Sala [b], Alex Aguilar [a], Catherine Tsangaris [c], Nikoletta Digka [c], Helen Kaberi [c], Ethel Eljarrat [b], Asunción Borrell [a]

[a] *Institute of Biodiversity Research (IRBio) and Department of Evolutionary Biology, Ecology and Environmental Sciences, Universitat de Barcelona, Barcelona, Spain*

[b] *Water, Environment and Food Chemistry, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain*

[c] *Institute of Oceanography, Hellenic Centre for Marine Research, Athens, Greece*

HIGHLIGHTS

- OPFRs were analysed in the muscle of bogues from the NW Mediterranean Sea.
- OPFR concentrations were higher in fish from the area off the city of Barcelona.
- No relationship was detected between OPFR levels and microplastic ingestion.
- Tri-n-butyl phosphate (TNBP) was the most abundant OPFR in both studied areas.
- ΣOPFRs ranged from nd to 1,194 ng g^{-1} lipid weight basis.

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ABSTRACT

Plastic litter pollution is increasing in the seas and oceans worldwide, raising concern on the potential effects of plasticizer additives on marine fauna. In this study, muscle samples of 30 bogues (*Boops boops;* Linneaus, 1758) from the North Western Mediterranean Sea were analysed to assess the concentrations of 19 organophosphate flame retardant (OPFR) compounds and to inspect any relationship with microplastic ingestion and relative levels of anthropization. Out of the 19 OPFRs analysed, 6 compounds were detected, being tri-n-butyl phosphate (TNBP), 2-ethylhexyldiphenyl phosphate (EHDPP) and tri-phenylphosphine oxide (TPPO) the most abundant. As expected, OPFR concentrations were higher in samples collected off the most anthropized area of the city of Barcelona than in those from the Cap de Creus Marine Protected Area, while no significant correlation was detected between OPFR concentrations and microplastic ingestion. The results of this manuscript provide a first evidence of OPFR presence in the muscle of the bogue and identify the coastal area off Barcelona as a possible concentration area for contaminants, further supporting the use of the bogue as an indicator species of plastic pollution in the Mediterranean Sea.

1. Introduction

Marine litter pollution has been raising major concerns in recent years due to its potential impact to marine biodiversity, particularly in the Mediterranean Sea, which is one of the most polluted seas worldwide (Suaria and Aliani, 2014). Microplastics (*i.e.*, plastics < 5 mm; Arthur et al., 2009) of primary origin, or derived from the degradation of larger plastic items, have been found in concentrations up to 115,000–1,050,000 particles km^{-2} in the NW Mediterranean Sea (UNEP/MAP, 2015). Ingestion of macro and microplastics has been reported in various species of marine birds (*e.g.*, Ryan et al., 2016), cetaceans (*e.g.*, Besseling et al., 2015), marine turtles (*e.g.*, Domènech et al., 2019) and fish (*e.g.*, Boerger et al.,

* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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2010; Garcia-Garin et al., 2019), with consequences ranging from severe injuries, to the obstruction of the digestive tract and, eventually, death by malnutrition (Gall and Thompson, 2015). Moreover, microplastics may act as vectors for the transport of inorganic and organic contaminants, which might cause toxic effects to the organism ingesting them (Rios et al., 2007).

Although plastic is inert (Galgani et al., 2013), the additives used to improve its features (*e.g.*, plasticizers, flame retardants) might modify its reactivity, producing toxic effects (Lithner et al., 2011). Indeed, high concentrations of plasticizers and flame retardants such as phthalates, polybrominated diphenyl ethers (PBDEs) and organophosphate flame retardants (OPFRs) may cause endocrine and carcinogenic effects on marine fauna (Aznar-Alemany et al., 2019; Du et al., 2019; Fossi et al., 2016).

Since the prohibition of PBDEs by the Stockholm Convention in 2009 (Stockholm-Convention, 2010), the use of OPFRs has increased exponentially (Pantelaki and Voutsa, 2019). Occurrence of these compounds has been studied in fresh water, air, sediment, biota, humans and some categories of food for human consumption (Du et al., 2019; Hou et al., 2016; Pantelaki and Voutsa, 2019; Zhao et al., 2019), but data regarding their toxicity is still limited. Some OPFRs, such as tris(chloroethyl) phosphate (TCEP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) were proven to be neurotoxic and carcinogenic (van der Veen and de Boer, 2012). *In vitro* studies on cells of experimental animals also showed tri-n-butyl phosphate (TNBP) and tris(phenyl) phosphate (TPHP) to cause developmental neurotoxicity, as well as adverse transcriptomic, reproductive, endocrine and carcinogenic effects (Bruchajzer et al., 2015; Du et al., 2019; Su et al., 2014; van der Veen and de Boer, 2012). Furthermore, 2-ethylhexyldiphenyl phosphate (EHDPP) showed adverse effects on female reproduction and foetal development in humans (Hu et al., 2017), and cytotoxic and transcriptomic effects in chicken embryonic hepatocytes, altering mRNA expression levels of multiple genes associated with different biological pathways (Shen et al., 2019). *In vitro* tests on human nuclear receptors also showed that several OPFRs may have potential endocrine disrupting effects (Kojima et al., 2013). Other novel OPFRs such as triphenylphosphine oxide (TPPO), whose occurrence has been reported in several environmental matrices (*e.g.*, Wang et al., 2017; Zhao et al., 2019) and biota (*e.g.*, Garcia-Garin et al., 2020) have effects still unknown on biota and humans.

The dramatic increase of production and use of OPFRs urges for a better assessment of their levels in the biota and of their potential effects on human health. The need for monitoring these contaminants is also stressed within the EU Marine Strategy Framework Directive (MSFD), which addresses the issue of marine pollution by litter and chemical contaminants through Descriptors 8, 9 and 10. According to the MSFD requirements, the good environmental status of the Mediterranean Sea would be reached when *concentrations of contaminants are at levels not giving rise to pollution effects* (D8), *contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards* (D9) and *properties and quantities of marine litter do not cause harm to the coastal and marine environment* (D10) (Zampoukas et al., 2014). In response to such requirements, the bogue (*Boops boops*; Linneaus, 1758) has been proposed as an indicator species of microplastic pollution in the Mediterranean Sea (Bray et al., 2019; Garcia-Garin et al., 2019).

The bogue is a benthopelagic species distributed across a wide latitudinal range in the eastern Atlantic Ocean, from Norway to Angola. It is common in the Mediterranean Sea, where it is ubiquitously distributed (FAO, 2020), and it is an edible species, what makes it relatively easy to collect for sampling. Finally, in the relatively small gut of this species, microplastics have been detected at high frequencies (Bray et al., 2019), further supporting the validity of this species as indicator for microplastic pollution.

The present study aims to assess the concentrations of OPFR compounds in the bogue and their relationship with microplastic ingestion and the relative levels of anthropization of the sampling areas, in order to test the potential use of the bogue as an indicator species for OPFR pollution. For this purpose, OPFRs were analysed in the muscle of bogues with known levels of microplastics (MP) in the gastrointestinal (GI) tract (Garcia-Garin et al., 2019), sampled from two areas in the North Western Mediterranean Sea characterized by different levels of urbanization and industrialization.

2. Materials and methods

2.1. Sample collection

The 30 bogues analysed in the current study were selected from a group of 102 individuals previously analysed for microplastic ingestion (Garcia-Garin et al., 2019). The selected sample included half individuals with microplastics in their GI tract and half without.

All bogues were caught during spring 2018 by local fishermen off the Spanish Catalan coast, in two areas characterized by a different degree of industrialization and urbanization: 1) an area in the vicinity of the city of Barcelona ($n = 15$); and 2) a marine protected area “the Cap de Creus (MPA)” ($n = 15$) (Fig. 1). The number of samples analysed for each area was selected to have a balance between the costs of the analysis and to have enough samples to perform statistical analysis. Sample collection was carried out avoiding contact with plastic material. Weight and total length of each fish were measured before storing at −20 °C. (Table 1).

2.2. Microplastic analysis

As previously mentioned, the analysis of microplastic ingestion was performed within the study by Garcia-Garin et al. (2019), which can be referred to for a detailed description of the methods used. Following the protocol developed in the framework of the MEDSEALITTER project (MEDSEALITTER consortium, 2019), the GI tract of the fish was dissected and digested with hydrogen peroxide (15%), and the microplastics in the digested filtrate were counted and classified by size, colour and type. Microplastic abundance was expressed as the number of microplastic items per individual.

2.3. OPFRs analysis

2.3.1. Standards and reagents

Nineteen OPFRs were analysed in the present study (Table S1). Analytical and labelled standards were obtained from different companies, as described in Giulivo et al. (2016). In addition, triethyl phosphate (TEP) and tri-n-propyl phosphate (TnPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-isopropylphenyl diphenyl phosphate (2IPPDPP), 4-isopropylphenyl diphenyl phosphate (4IPPDPP) and bis(4-isopropylphenyl) phenyl phosphate (B4IPPPP) were obtained from Wellington Lab-oratories Inc. (Guelph, ON, Canada).

2.3.2. Sample preparation

Muscle samples of about 15 g were lyophilised during 48 h. After that, samples were prepared according to Giulivo et al. (2016): 0.5 g dry weight (dw) were extracted by sonication using 15 mL of hexane:acetone (1:1) during 15 min. The extraction was carried out twice, and both extracts were combined. Then, the extract was evaporated under a gentle nitrogen stream in order to change the solvent, and it was reconstituted in 5 mL of hexane:methanol (1:3).

Fig. 1. Study area showing the two sampling areas off Barcelona and Cap de Creus MPA.

Table 1
Sample location, biometric data, number of MP in the GI tract and ΣOPFR concentrations in the muscle of bogue specimens.

ID	Area	Total length (mm)	Weight (g)	MP (items individual^{-1})	ΣOPFR (ng g^{-1} lw)
BB13	Barcelona	155	39.3	4	1,443
BB15	Barcelona	175	54.0	3	710
BB16	Barcelona	185	62.9	3	475
BB12	Barcelona	170	48.9	3	328
BB7	Barcelona	175	59.2	2	309
BB14	Barcelona	160	47.9	2	332
BB4	Barcelona	175	63.4	2	353
BB6	Barcelona	160	43.9	1	242
BB26	Barcelona	210	81.0	0	557
BB8	Barcelona	170	45.8	0	250
BB27	Barcelona	235	135.4	0	876
BB2	Barcelona	195	75.9	0	95.7
BB28	Barcelona	215	83.7	0	2,566
BB24	Barcelona	235	122.4	0	1,310
BB3	Barcelona	185	65.3	0	234
BM11	Cap de Creus MPA	220	146.6	3	162
BM3	Cap de Creus MPA	220	124.7	2	851
BM17	Cap de Creus MPA	235	127.2	2	1,379
BM8	Cap de Creus MPA	300	336.2	2	470
BM5	Cap de Creus MPA	300	411.7	1	904
BM1	Cap de Creus MPA	220	128.0	1	nd
BM12	Cap de Creus MPA	190	98.7	1	210
BM2	Cap de Creus MPA	240	162.9	0	177
BM29	Cap de Creus MPA	240	152.4	0	nd
BM26	Cap de Creus MPA	245	141.6	0	nd
BM19	Cap de Creus MPA	215	105.2	0	nd
BM18	Cap de Creus MPA	220	99.4	0	531
BM27	Cap de Creus MPA	220	126.3	0	319
BM31	Cap de Creus MPA	255	192.0	0	16
BM30	Cap de Creus MPA	260	191.9	0	962

nd: below detection limits.

The solution was centrifuged for 10 min at 4000 rpm and an aliquot of 200 μL was analysed by turbulent flow chromatography (TFC) coupled with LC-MS/MS. Labelled OPFR standards were added prior to analysis. Lipid weight (lw) was determined gravimetrically from the remaining 4.8 mL, after evaporating the solvent using a nitrogen stream and drying the sample in an oven at 90 °C until constant weight was reached.

2.3.3. Instrumental analysis

Purification was done according to Giulivo et al. (2016), and it was performed on-line at the beginning of the instrumental analysis with a Thermo Scientific TurboFlow™ system. Columns used for purification were CycloneTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm). An analytical column (Purosphere Star RP-18, 125 mm × 0.2 mm) was used for chromatographic separation.

The mobile phase was a gradient of water (0.1% formic acid) and methanol (ammonium acetate) at 0.75 mL min^{-1} (Santín et al., 2016). Mass spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source. For all compounds, selective reaction monitoring (SRM) mode was used with two transitions selected for each analyte.

2.3.4. Quality assurance

A blank was included every 10 samples. If its signal did not exceed 10% of the sample batch signals, its OPFR levels were subtracted from the corresponding batch of samples. If, on the contrary, the blank signal was higher than 10% of the sample batch signals, all samples in the batch were re-analysed. All the non-volumetric material was heated at 340 °C and rinsed with the appropriate solvent before use, and no plastic material was used to avoid contamination. Recoveries were 48–102% with RSDs between 0.3 and 24.7%. Limits of detection (LODs) and limits of quantification (LOQs) were 0.2–19.3 ng g^{-1} lw and 1.0–24.8 ng g^{-1} lw, respectively.

2.3.5. Expression of concentrations

OPFRs are lipophilic compounds and, as such, their concentrations are usually expressed on a lipid weight basis to normalize for varying lipid content between species, individuals and tissues (Krahn et al., 2003).However, here we express the concentrations of OPFRs on three bases to allow comparison with previous studies: extractable lipid basis (lw), wet weight basis (ww), and dry weight basis (dw).

2.4. Statistical analysis

The sum of all OPFR compounds (ΣOPFR) was calculated excluding the compounds that were below the limit of detection. The normality and heteroscedasticity of the distribution of ΣOPFR concentrations were tested using the Shapiro Wilk and the Levene test, respectively. ΣOPFR concentrations did not follow a normal distribution ($p < 0.05$, Shapiro Wilk test), although variances were homogeneous ($p = 0.28$, Levene test). Thus, ΣOPFR concentrations were normalized using the square root. Data were checked for possible collinearity between variables and the presence of outliers (Zuur et al., 2010). ΣOPFR concentrations were modelled using GLMs (generalized linear models) fitted with a Gaussian distribution. The explanatory variables used for building the models included: the level of anthropogenic impact, categorized as low (MPA) and high (off Barcelona); the number of microplastics (MP) detected in the fish GI tract; the fish length and the fish weight. Best fitting models were selected using the Akaike information criteria corrected for small sample sizes (AICc; Hurvich and Tsai, 1989) and the corresponding AICc increments (ΔAICc) and weights (AICc wt; Johnson and Omland, 2004). Differences in the OPFR concentrations, which were related to the level of anthropogenic impact and the presence or absence of microplastics in the fish GI tract, were highlighted through a Principal Component Analysis (PCA). The significance level was set at $p < 0.05$. R.3.6.2. statistical software was used for all analyses (R Core Team, 2018).

3. Results

Microplastic abundance ranged from 0 to 4 items $individual^{-1}$ in the bogues sampled in the area off Barcelona and from 0 to 3 items $individual^{-1}$ in those sampled in the Cap de Creus MPA (Table 1). Mean microplastic abundance was slightly higher in the bogues sampled off Barcelona than in those sampled in the Cap de Creus MPA (1.33 ± 1.45 and 0.8 ± 1.0 items $individual^{-1}$, respectively).

OPFRs were detected in all the bogues sampled off Barcelona and in 11 out of the 15 sampled in the Cap de Creus MPA. ΣOPFR concentrations ranged from below LOD (nd) to 2,566 ng g^{-1} lw, and were higher (96–2,566 ng g^{-1} lw) in the bogues sampled off Barcelona than in those sampled in the Cap de Creus MPA (nd to 1,379 ng g^{-1} lw) (Table 1).

The distribution of the different OPFR compounds (lw basis) in each area are depicted in Fig. 2. Among the 19 OPFRs analysed, 6 were detected in the bogues muscle. TNBP, EHDPP and TPPO were the most abundant compounds, detected in 100, 40 and 27% of the bogues sampled off Barcelona and in 67, 7 and 13% of the bogues sampled in the Cap de Creus MPA, respectively. Tri-n-propyl phosphate (TPP) and TPHP were detected in 47 and 7% of the samples from Barcelona, while they were not detected in the samples from the MPA. On the contrary, TDCIPP was detected only in 7% of the samples from the MPA (Table 2).

The mean and standard deviation of the concentration of each compound and of the ΣOPFR are shown in Table 2, grouped by sampling area. Mean ΣOPFR concentration, expressed on a lipid weight basis, was 672 (SD = 657) ng g^{-1} in the samples from Barcelona and 379 (SD = 411) ng g^{-1} in the samples from Cap de Creus MPA (Table 2).

A total of 16 different GLMs were fitted from the combination of the 4 variables (anthropogenic impact, number of microplastics, length and the weight of the fish) plus the level of anthropogenic impact*MP interaction (Table 3). The best fitting model showed a significant correlation of ΣOPFR concentrations with anthropogenic impact and fish length but the correlation with number of ingested microplastics was not significant (Table 4). No correlation between ΣOPFR concentrations and fish weight was found, as the latter was not included in the model (Table 3).

PCA ordination of samples considering the six OPFRs detected in the muscle of bogues and the two factors "area" and "microplastic presence in the GI tract" produced a two-dimensional pattern, with the first two components explaining 54.1% of the total variance (Fig. 3). Despite some overlapping, most of the separation between sampling areas and levels of microplastic ingestion, occurred along PC1 axis, mainly referring to EHDPP (0.60) and TPPO (0.57). On the other side, TNBP (0.71) and TDCIPP (−0.62) determined most of the separation along PC2.

4. Discussion

In this study, the levels of several plasticizers and flame

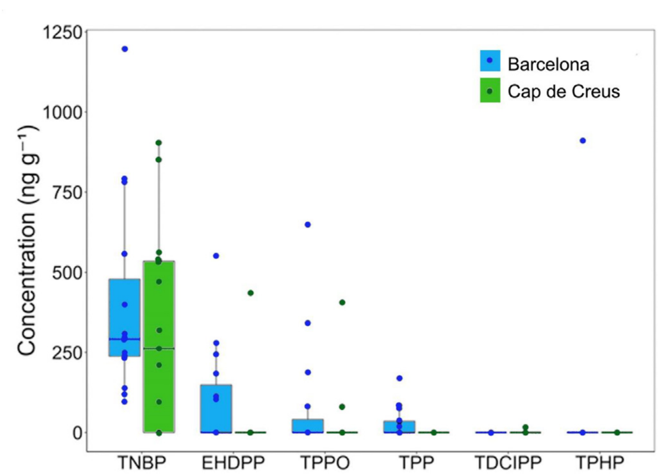

Fig. 2. Box-plots illustrating the OPFR concentrations (lw) detected in the bogues sampled off Barcelona and in the Cap de Creus MPA.

Table 2
Individual OPFR concentrations (mean ± SD, expressed in ng g^{-1} ww, dw and lw) and frequency of detection measured in the muscle of bogues collected from the area off Barcelona and in the Cap de Creus MPA.

	Barcelona				Cap de Creus MPA			
	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})	Frequency of detection (%)	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})	Frequency of detection (%)
TPPO	1.0 ± 1.8	4.1 ± 7.0	84.0 ± 183	27	0.7 ± 1.7	2.0 ± 5.3	40.5 ± 117	13
TPP	0.5 ± 0.5	1.8 ± 2.0	29.2 ± 47.7	47	nd	nd	nd	0
TDCIPP	nd	nd	nd	0	0.1 ± 0.3	0.3 ± 1.1	1.08 ± 4.2	7
TPHP	0.8 ± 3.1	3.4 ± 13.2	60.7 ± 235	7	nd	nd	nd	0
TNBP	6.5 ± 1.6	26.1 ± 6.3	400 ± 307	100	7.0 ± 6.4	25.4 ± 23.1	316 ± 313	67
EHDPP	1.9 ± 2.6	7.3 ± 9.8	98.5 ± 159	40	0.3 ± 1.1	1.1 ± 4.2	29.0 ± 112	7
ΣOPFRs	10.6 ± 4.9	42.6 ± 19.4	672 ± 657	100	7.9 ± 6.5	28.8 ± 23.5	379 ± 411	73

nd: below detection limits.

Table 3
GLM results for ΣOPFR concentrations in the bogues muscle, ranked by the Akaike information criteria corrected for small sample sizes (AICc). The variables included in the models were: level of anthropogenic impact (low and high), number of microplastics in the fish GI tract (MP), fish length (mm) and fish weight (g). The best-fit model is shown in bold. df = number of parameters; ΔAICc = AICc increments, AICc wt = AICc weights.

	Model	df	AICc	ΔAICc	AICc wt
M1	**Level of anthropogenic impact + MP + Fish length**	**5**	**235**	**0.0**	**0.18**
M2	Level of anthropogenic impact + Fish length	4	236	0.5	0.14
M3	Level of anthropogenic impact + MP	4	237	1.3	0.10
M4	Level of anthropogenic impact	3	237	1.4	0.09
M5	Level of anthropogenic impact + MP + Fish length + Fish weight	6	238	2.3	0.06
M6	Level of anthropogenic impact + MP + Fish weight	5	238	2.9	0.04
M7	Level of anthropogenic impact + Fish weight	4	239	3.0	0.04
M8	Level of anthropogenic impact + Fish length + Fish weight	5	239	3.4	0.03
M9	MP	3	239	3.4	0.03
M10	Level of anthropogenic impact * MP + Fish length + Fish weight	7	240	4.3	0.02
M11	Fish length	3	241	5.3	0.01
M12	Fish weight	3	241	5.3	0.01
M13	MP + Fish length	4	242	5.9	0.01
M14	MP + Fish weight	4	242	6.1	0.01
M15	Fish length + Fish weight	4	244	7.9	0.00
M16	MP + Fish length + Fish weight	5	244	8.5	0.00

Table 4
Summary of the outputs of the best-fit GLM, including the variables "level of anthropogenic impact", "MP" and "Fish length" (M1).

Term	Coefficient estimate	Standard error	Z value	Pr(>|z|)
Intercept	−13.89	15.22	−0.91	0.37
Level of anthropogenic impact (Low)	−16.19	5.42	−2.99	<0.01
MP	3.19	1.80	1.77	0.09
Fish length	0.18	0.08	2.38	0.02

retardants of the OPFR family were analysed in the muscle of bogues sampled from two areas subject to different anthropogenic pressures, and the relationship between microplastic ingestion and OPFR concentrations was investigated. Three main results were obtained: 1) mean ∑OPFR concentrations were higher in the most anthropized area off Barcelona, 2) positive correlation was found between ∑OPFR concentrations and fish length, and 3) the correlation between ∑OPFR concentrations and the abundance of ingested MP was not significant.

Flame retardants and plasticizers have been detected in fresh water, air, sediment, humans and biota (Du et al., 2019; Garcia-Garin et al., 2020; Hou et al., 2016; Pantelaki and Voutsa, 2019). As most OPFRs can easily be metabolized (WHO, 1990, 1991, 1997, 1998, 2000), the constant presence of these compounds in all the environmental compartments would point out to permanent emissions and exposure and possibly negative effects to humans and biota.

Information on the presence of OPFRs in aquatic biota is scarce and only in the last few years these compounds have been analysed in fish. Thus, studies reporting OPFR concentration in freshwater fish are few. Giulivo et al. (2017) analysed fish from the Adige, Sava and Evrotas rivers (Mediterranean Sea), finding lower OPFR levels than those reported in the present study. On the contrary, Santín et al. (2016), who analysed OPFR in fish from the Llobregat river, which flows into the Mediterranean Sea near Barcelona, reported concentrations in the same range as our results. Although they found that IPPP (a compound not detected in the bogues analysed in the present study) was the most concentrated OPFR, they also detected EHDPP (63 ± 165 ng g^{-1} lw) as one of the most concentrated OPFR in fish, consistently with our study.

Even more limited information is available on the occurrence of OPFR in marine fish, which, to the best of our knowledge, has been reported only in 5 published articles (Brandsma et al., 2015, Giulivo et al., 2016; Hallanger et al., 2015; Kim et al., 2011; Sundkvist et al., 2010), none of them referring to the Mediterranean Sea. OPFR concentrations in the bogues analysed in the current study are higher than the OPFR concentrations found in herring (*Clupea harengus*) from the sea of Sweden, reported by Sundkvist et al. (2010), in benthic and pelagic fish species from Western Shetland, reported by Brandsma et al. (2015), and in salmon (*Salmo salar*)

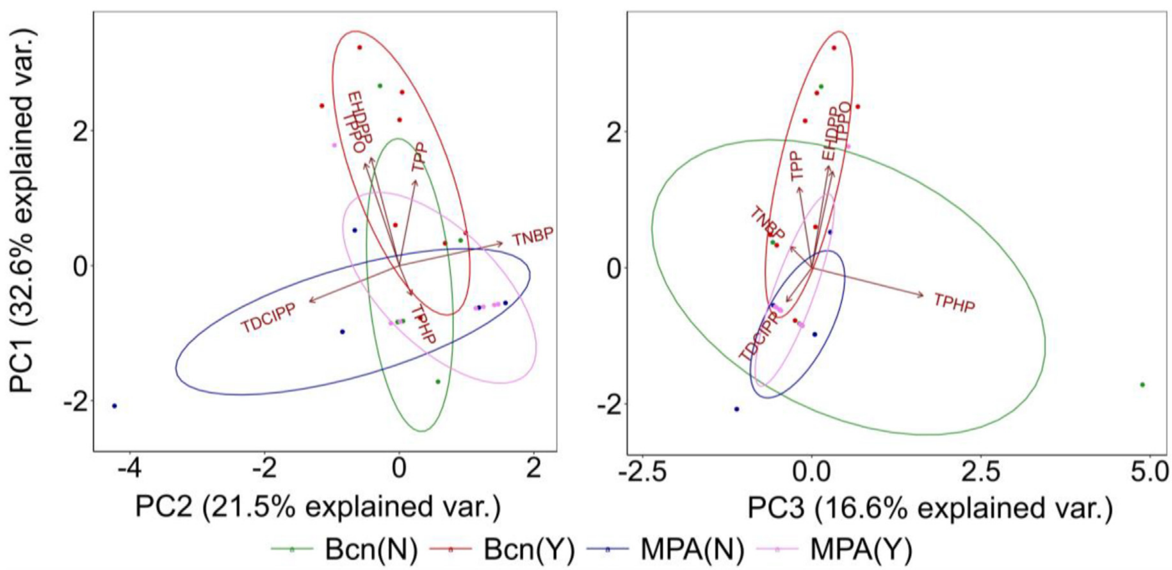

Fig. 3. PCA analysis of OPFR concentrations for area (Barcelona (Bcn) and Cap de Creus MPA (MPA)) and presence (Y)/absence (N) of microplastics inside the bogues GI tract.

from the Atlantic, reported by Giulivo et al. (2016). The above comparisons suggest that the Mediterranean Sea is more polluted in terms of OPFR concentrations than the Baltic Sea, the North Sea or the Atlantic Ocean, which can be indicative of higher plastic inputs in the basin. On the other hand, OPFR concentrations in the bogues sampled from the Barcelona area are in the same order of magnitude as those reported by Kim et al. (2011) in fish samples from the Philippines, in which TNBP was one of the most abundant OPFR, like in the present study. Finally, concentrations of TDCIPP, TPHP and EHDPP in capelin (*Mallotus villotus*) from the high-arctic archipelago of Svalbard (Hallanger et al., 2015) were higher than those found in the Mediterranean bogues. These studies indicate that OPFRs are omnipresent in the marine environment, although their compound composition and concentrations vary among different geographic areas.

$\sum$OPFR concentrations were significantly different in the bogues from the two sampling areas, being higher, as expected, in those collected from the most anthropized area. Barcelona is the second city of the Mediterranean Sea in terms of estimated inputs of plastic, with an estimate annual input of plastic litter of 1,800 tons (Liubartseva et al., 2018). Its littoral hosts many industries, large commercial and tourist ports, and the city is located between the rivers Besòs and Llobregat, where wastewater treatment plants may contribute to the input of plastics in the sea. The importance of sewage plants as source of OPFR contamination was stressed by Sundkvist et al. (2010), who found OPFR concentrations two times higher in fish from lakes close to sewage treatment plants than in fish from other lakes. These factors might explain the higher OPFR concentrations in the bogues sampled from the area off Barcelona than in those sampled from Cap de Creus MPA. Furthermore, PCA analysis revealed that differences between sampling areas were mainly due to TPP and TPHP, which were detected only in Barcelona, and TDCIPP, which was detected only in Cap de Creus MPA. In addition, the concentrations of EHDPP and TPPO also separated the two sampling areas along the PC1 axis. Differences in the concentrations of specific congeners might be due to pollution by different types of plastics in the two sampling areas. However, further investigations are needed to allow relating each OPFR congener to its marine litter source.

Significant positive correlations were detected between $\sum$OPFR and fish length, although no significant correlation was found between $\sum$OPFR and fish weight. We might expect a similar relationship of $\sum$OPFR concentrations with fish length and weight, although fish length and weight are not linearly correlated, but their relationship follow instead an exponential function. While some studies do not report any significant correlation between $\sum$OPFR concentrations and fish length or weight (Kim et al., 2011; Malarvannan et al., 2015), others do: Choo et al. (2018), similarly to our results, reported positive correlation between TNBP concentrations in the muscle of crucian carp and the fish length and weight, implying that TNBP may accumulate in the muscle as the fish grows. Nevertheless, most OPFRs can easily be metabolized (WHO, 1990, 1991, 1997, 1998, 2000) and further research is needed to understand whether OPFRs accumulate in fish or biomagnify through the food web.

The number of ingested microplastics was included in the best GLM but it was not significantly correlated to the $\sum$OPFR concentration in the fish muscle. Similarly, PCA analysis did not reveal any difference between the concentrations of OPFR congeners and the presence of microplastics in the bogues GI tract, although one would expect the presence of microplastics to be highly related to the levels of OPFRs, which are widely used as plasticizers (Du et al., 2019). However, microplastics are likely to remain in the fish GI tract only few hours/days, depending on its length and thus on the digestion transit time (German and Horn, 2006), limiting the time during which additives could be absorbed by the fish tissues. In addition, OPFRs can also reach the fish tissues through the direct contact of the gills and the epidermis with the water and not only from microplastic ingestion (Kim et al., 2011). These aspects may explain why the abundance of ingested microplastics was not correlated to the $\sum$OPFR concentrations in the muscle of the bogues.

There is scarce data on potential toxic effects caused by the OPFRs commonly used as flame retardants and plasticizers. Among the OPFR compounds detected in the bogue samples, the effects related to TNBP, TDCIPP, and TPHP are the most studied. *In vitro* studies of OPFR effects on human cells have shown that TNBP, at concentrations ranging from 0 to 43 $\mu g\ ml^{-1}$, could inhibit cell viability, overproduce reactive oxygen species, induce DNA lesions, and increase the lactate dehydrogenase leakage (An et al., 2016). Other investigations showed that TDCIPP, at concentrations higher than 2 $\mu g\ ml^{-1}$, caused apoptosis in human corneal epithelial cells

(Xiang et al., 2017). On the other hand, *in vivo* toxicological laboratory tests have shown that TNBP, TDCIPP and TPHP were lethal for certain animal species, mainly rats, chick, rabbits, zebrafish and mice, with a median lethal dose (LD_{50}) of over 1,400 μg g^{-1} (Du et al., 2019; van der Veen and de Boer, 2012). ∑OPFR found in the muscle of bogues ranged from nd to 18.2 ng g^{-1} ww, which are values five orders of magnitude lower than those referred to as LD_{50} in laboratory animals. We can conclude that, at the concentration found in our study, the potential toxic impacts of OPFRs on bogues are limited, although the assessment of their effect should be made at a medium – large temporal scale as they may lead to chronic toxicity.

To determine the potential risk caused by the levels of OPFRs reported in fish to the human population, the intake of OPFRs through fish consumption was estimated considering that all edible fish would have concentrations similar to those found in the bogue. In the present study, the mean ∑OPFR concentration detected in bogues was of 9.27 ng g^{-1} (ww), which would imply, considering that the annual fish consumption in Europe is 22.5 kg (FAO, 2018) and the average human body weight is 60 kg, a mean OPFR intake by humans of 9.5 ng kg^{-1} day^{-1}. Such OPFR intake due to the consumption of Mediterranean bogues would be two to three orders of magnitude lower than the reference doses of TNBP and TPHP, which are 2,400 and 7,000 ng kg^{-1} day^{-1}, respectively (Van den Eede et al., 2011). In addition, the total dietary intake of 9.5 ng kg^{-1} day^{-1} is also much lower than the suggested guideline value of 40 μg kg^{-1} day^{-1} for the sum of TNBP, TBEP, TCEP, TCPP, TEHP and TPP (Sundkvist et al., 2010). Nevertheless, this dose refers only to fish intake and we should notice that there is also a large risk of exposure to OPFRs via other kinds of food intake (Li et al., 2019; Zhao et al., 2019) and via inhalation (Marklund et al., 2003). Thus, the concentrations detected in bogues are not negligible, and due to the global increase of plastic litter pollution, OPFR concentrations in marine organisms will likely increase, mostly affecting organisms and ecosystems close to highly anthropized areas. As the fish consumption in the world is constantly rising (FAO, 2018), the potential risks to human health are also expected to rise, urging for further research on the potential toxic effects of OPFRs.

5. Conclusions

The results of this study provide a first evidence of OPFR presence in the muscle of the bogue, identifying the waters off Barcelona as a potential area of concentration for these pollutants. The relationship between OPFR concentrations and microplastic ingestion is unclear, as no significant correlation was found between these two variables. The current OPFR concentrations in the bogue do not seem to be harmful to humans. However, dietary intake estimation should be conducted based on a broader spectrum of foodstuff samples and not just on one fish species. Further research is needed to analyse the occurrence of OPFRs in other edible species and their potential toxic effects in fish and, thus, indirectly, on human health. However, our results contribute to increase the knowledge on the levels of OPFRs in marine biota, highlighting the bogue as good indicator species of OPFR pollution in the Mediterranean Sea.

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.

CRediT authorship contribution statement

Odei Garcia-Garin: Conceptualization, Resources, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Morgana Vighi:** Writing - original draft, Writing - review & editing, Supervision. **Berta Sala:** Methodology, Validation, Supervision. **Alex Aguilar:** Supervision, Project administration, Funding acquisition. **Catherine Tsangaris:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Supervision, Project administration, Funding acquisition. **Nikoletta Digka:** Methodology, Validation, Resources, Writing - original draft, Supervision. **Helen Kaberi:** Resources, Writing - original draft, Project administration, Funding acquisition. **Ethel Eljarrat:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Supervision, Project administration, Funding acquisition. **Asunción Borrell:** Conceptualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126569.

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3.2. Organophosphate contaminants in North Atlantic fin whales

Autores: Odei Garcia-Garin[1], Berta Sala[2], Alex Aguilar[1], Morgana Vighi[1], Gísli A. Víkingsson[3], Valerie Chosson[3], Ethel Eljarrat[2], Asunción Borrell[1]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences, and Institute of Biodiversity Research (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain
[2]Water, Environmental and Food Chemistry, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[3]Marine and Freshwater Research Institute, P. O. Box 1390, Skúlagata 4, 121 Reykjavík, Iceland

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Abstract:
Pollution of the marine environment by litter composed of plastics is a growing concern. Chemical additives such as organophosphate flame retardants (OPFRs),which are added to plastics to improve their qualities, are in focus because they allegedly cause adverse effects on marine fauna. Here we analyse OPFR levels in the muscle of fin whales because, as a mysticete, this cetacean obtains its food by filter-feeding and is thus highly vulnerable to marine litter. Moreover, the fin whale performs long-range migrations from low-latitude areas in winter to high latitude areas in summer, a trait that makes it a potentially good large-scale biomonitor of pollution. We also analyse OPFR levels in its main prey, the krill *Meganyctiphanes norvegica*, to assess transfer through diet. The samples analysed consisted of muscle tissue from20 fin whales and whole-body homogenates of 10 krill samples, all collected off West Iceland. From the 19 OPFRs analysed, we detected 7 in the fin whale and 5 in the krill samples. Tri-n-butyl phosphate (TNBP), Isopropylated triphenyl phosphate (IPPP) and Triphenylphosphine oxide (TPPO) were the most abundant compounds found in both species. Mean ΣOPFR concentration, expressed on a lipid weight basis, was 985 (SD = 2239) ng g−1 in fin whale muscle, and 949 (SD = 1090) ng g−1 in krill homogenates. These results constitute the first evidence of the presence of OPFRs in the tissues of fin whales. Furthermore, they seem to support the non-significance of bioaccumulation of OPFRs through lifespan and of biomagnification trough the food web.

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Organophosphate contaminants in North Atlantic fin whales

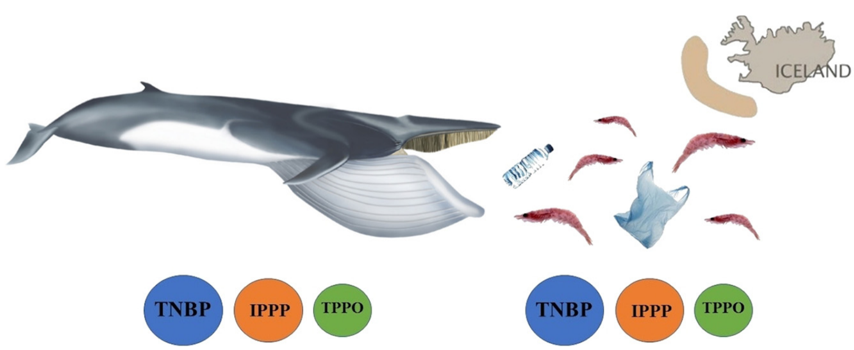
Odei Garcia-Garin [a], Berta Sala [b], Alex Aguilar [a], Morgana Vighi [a], Gísli A. Víkingsson [c], Valerie Chosson [c], Ethel Eljarrat [b,*], Asunción Borrell [a]

[a] Institute of Biodiversity Research (IRBio), Department of Evolutionary Biology, Ecology and Environmental Sciences, Universitat de Barcelona, Barcelona, Spain
[b] Water, Environmental and Food Chemistry, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Marine and Freshwater Research Institute, P. O. Box 1390, Skúlagata 4, 121 Reykjavík, Iceland

HIGHLIGHTS

- We provide the first evidence of OPFR presence in the tissues of fin whales.
- OPFRs were detected in the fin whale main prey, the krill *Meganyctiphanes norvegica*.
- TNBP, IPPP and TPPO are the most abundant OPFRs in both fin whales and krill.
- OPFRs do not seem to bioaccumulate or biomagnify.

GRAPHICAL ABSTRACT



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ABSTRACT

Pollution of the marine environment by litter composed of plastics is a growing concern. Chemical additives such as organophosphate flame retardants (OPFRs), which are added to plastics to improve their qualities, are in focus because they allegedly cause adverse effects on marine fauna. Here we analyse OPFR levels in the muscle of fin whales because, as a mysticete, this cetacean obtains its food by filter-feeding and is thus highly vulnerable to marine litter. Moreover, the fin whale performs long-range migrations from low-latitude areas in winter to high-latitude areas in summer, a trait that makes it a potentially good large-scale biomonitor of pollution. We also analyse OPFR levels in its main prey, the krill *Meganyctiphanes norvegica*, to assess transfer through diet. The samples analysed consisted of muscle tissue from 20 fin whales and whole-body homogenates of 10 krill samples, all collected off West Iceland. From the 19 OPFRs analysed, we detected 7 in the fin whale and 5 in the krill samples. Tri-n-butyl phosphate (TNBP), Isopropylated triphenyl phosphate (IPPP) and Triphenylphosphine oxide (TPPO) were the most abundant compounds found in both species. Mean $\sum$OPFR concentration, expressed on a lipid weight basis, was 985 (SD = 2239) ng g^{-1} in fin whale muscle, and 949 (SD = 1090) ng g^{-1} in krill homogenates. These results constitute the first evidence of the presence of OPFRs in the tissues of fin whales. Furthermore, they seem to support the non-significance of bioaccumulation of OPFRs through lifespan and of biomagnification trough the food web.

* Corresponding author.
E-mail address: eeeqam@cid.csic.es (E. Eljarrat).

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1. Introduction

Marine litter, and particularly that composed of plastics, is increasing in oceans worldwide (*e.g.*, Lebreton et al., 2018). The physical adverse effects of this type of pollution on marine fauna are well known and include entanglement, suffocation, and obstruction of the digestive system (*e.g.*, De Stephanis et al., 2013; Di-Méglio and Campana, 2017). However, chemical effects related with the ingestion of plastics have been mostly associated with the toxicity of plasticizers and are still under assessment (Hermabessiere et al., 2017). Among the constituents of plastics, polybrominated diphenyl ethers (PBDEs), phthalates and organophosphate flame retardants (OPFRs) have been claimed to induce organic damage to marine fauna (Aznar-Alemany et al., 2019; Fossi et al., 2016). PBDEs were banned by the Stockholm Convention in 2009 (Stockholm-Convention, 2010) and, since then, the production and use of OPFRs as alternative flame retardants and plasticizers has dramatically increased (Pantelaki and Voutsa, 2019).

The effects of exposure to OPFRs are poorly understood, although some of these compounds, such as tri-n-butyl phosphate (TNBP) and tris(phenyl) phosphate (TPHP), have been observed to cause developmental neurotoxicity and to have endocrine, carcinogenic and adverse reproductive effects in experimental animals (Bruchajzer et al., 2015; van der Veen and de Boer, 2012). Toxicological studies on diphenylcresyl phosphate (DCP), another OPFR which is also used as flame retardant and plasticizer (van der Veen and de Boer, 2012), have elicited concern for the aquatic toxicity of this compound (Washington State, 2006). Sigma-Aldrich (2019) also pointed its potentially toxic impact on aquatic organisms because of alleged effects on reproduction and development (Washington State, 2006). Moreover, Chlorinated OPFRs are suspected carcinogens. In rats and mice they have been found to induce tumour growth in kidneys, liver and thyroid apparently associated to exposition to Tris(chloroethyl) phosphate (TCEP) and Tris(chloroisopropyl) phosphate (TCIPP), and to have similar effects in brain and testes, this time associated to exposition to Tris (1,3-dichloro-2-propyl) phosphate (TDCPP) (van der Veen and de Boer, 2012).

The occurrence of OPFRs has been investigated in detail in humans, air, sediment, freshwater and some terrestrial biota (Hou et al., 2016; Pantelaki and Voutsa, 2019), but there are few reports on the occurrence of OPFRs in the marine environment and, particularly, in marine organisms. Some studies have focused on OPFR occurrence in cetaceans (*e.g.*, Aznar-Alemany et al., 2019; Sala et al., 2019) although the scope of species analysed has been restricted to dolphins, which are top predators and often have a limited distribution range. OPFR occurrence in the long-ranging oceanic mysticetes, or baleen whales, has not so far been reported despite they obtain their food by filtering out small organisms from the water, a feature that makes them highly vulnerable to the ingestion of marine plastics.

The fin whale (*Balaenoptera physalus*; Linnaeus, 1758) is a cosmopolitan mysticete that performs annual migrations from low-latitude breeding areas in winter to high-latitude feeding areas in summer (Aguilar and García-Vernet, 2018). During these long-range migrations, fin whales cross water masses that may be contaminated by different types of pollutants, including the ever-increasing plasticizers (*e. g.* Franeker and Law, 2015; Strobel et al., 2018). Consequently, they have the capability of integrating the incidence of these chemicals over large geographical scales averaging the heterogeneity of environmental local signals to which other organisms of more restricted distribution would be subject. From this perspective, fin whales may be considered potentially good indicator species to assess the presence of toxic compounds related to plastic pollution, such as OPFRs, in large oceanic bodies of water, like they have been used to monitor the chemo-physical characteristics of water masses (Borrell et al., 2018). In the North Atlantic Ocean, the fin whale has a broad distribution that extends from about 80° N (Svalbard archipelago) to almost the Equator (south of the islands of Cape Verde), including the Mediterranean and Baltic seas (Aguilar and García-Vernet, 2018). With a population estimated at about 70,000 individuals and structured in at least 7 discrete stocks (International Whaling Commission, 2009), it is one of the most abundant mysticetes in this Ocean.

The aim of this study was to investigate the presence and concentration of OPFRs in the fin whale and in its main prey, the krill *Meganyctiphanes norvegica*, off West Iceland, to assess the potential incidence of bioaccumulation and biomagnification processes.

2. Materials and methods

2.1. Sampling

Muscle samples were collected from fin whales caught by the Hvalur H/F whaling company off western Iceland (Denmark Strait) during the summer of 2015. Samples from 20 whales (13 males and 7 females) were analysed. The body length of these whales ranged from 16.8 to 20.4 m and their reproductive status was varied (Table 1). The reproductive status of females was established by examination of the ovaries and mammary glands and that of males through the histological examination of the testicle, as described by Lockyer (1984). Samples of the whale prey, consisting of different sets of samples of whole body homogenates of euphausiids, were collected from the forestomach (first chamber) of ten fin whales caught during the 2009–2013 summer seasons. The body size of the euphausiids and their aspect suggested that they belonged to the species *Meganyctiphanes norvegica*, which is the most abundant prey consumed by fin whales off western Iceland (Víkingsson, 1997). All samples were kept frozen until analysis.

2.2. Standards and reagents

A total of nineteen OPFRs were analysed in the present study. Analytical standards were purchased from different companies: Tris(2-butoxyethyl) phosphate (TBOEP), Tris(chloroethyl) phosphate (TCEP), Tris(chloroisopropyl) phosphate (TCIPP), Trihexyl phosphate (THP) and Tris(2-ethylhexyl) phosphate (TEHP) were obtained from Santa Cruz Biotechnology (SantaCruz, CA, USA). Isodecyldiphenyl phosphate (IDPP) and 2-ethylhexyldiphenyl phosphate (EHDPP) were purchased from AccuStandard (New Haven, CT, USA). Diphenylcresyl phosphate (DCP), Tri-*n*-butyl phosphate (TNBP), Triphenyl phosphate (TPHP), Triphenylphosphine oxide (TPPO), Tris(1,3-dichloro-2-propyl) phosphate (TDCPP), Triethyl phosphate (TEP) and Tri-n-propyl phosphate (TnPP) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Table 1
Biological variables and ∑OPFR concentrations (expressed in ng g^{-1} lipid weight (lw)) for each fin whale studied.

ID	Sex	Reproductive status	∑OPFRs
F15007	Male	Mature	270
F15017	Male	Mature	36.4
F15031	Male	Mature	824
F15049	Male	Mature	508
F15071	Male	Mature	1329
F15088	Male	Mature	162
F15144	Male	Mature	161
F15026	Male	Immature	10,232
F15034	Male	Immature	470
F15063	Male	Immature	31.9
F15085	Male	Immature	313
F15116	Male	Immature	216
F15151	Male	Immature	248
F15013	Female	Mature	507
F15025	Female	Mature	363
F15028	Female	Mature	87.0
F15015	Female	Immature	117
F15059	Female	Immature	1875
F15096	Female	Immature	1619
F15146	Female	Immature	339

Tricresyl phosphate (TMCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). 2-isopropylphenyl diphenyl phosphate (2IPPDPP), 4-isopropylphenyl diphenyl phosphate (4IPPDPP) and Bis (4-isopropylphenyl) phenyl phosphate (B4IPPPP) were purchased from Wellington Lab-oratories Inc. (Guelph, ON, Canada). Isopropyl phenyl phosphate (IPPP) was purchased from Chiron (Trondheim, Norway). Labelled d_{15}-TDCPP, d_{27}-TNBP, d_{12}-TCEP and $^{13}C_2$-TBOEP were obtained from Wellington Lab-oratories Inc. (Guelph, ON, Canada). Labelled d_{15}-TPHP was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

2.3. Sample preparation

Samples of approximately 10 g of frozen muscle and whole-krill homogenates were subject to lyophilisation for 48 h. Sample preparation was done according to Giulivo et al. (2016): 0.5 dry weight (dw) samples were extracted by sonication using 15 mL hexane:acetone (1:1) during 15 min. The extraction was carried out twice, and both extracts were combined. The resulting extract was reduced under a gentle nitrogen stream in order to change the solvent, and it was reconstituted in 5 mL hexane:methanol (1:3). The solution was centrifuged during 10 min at 4000 rpm and an aliquot of 200 μL was used for instrumental analysis. Lipid weight (lw) was determined gravimetrically from the remaining 4.8 mL by evaporating the solvent using a nitrogen stream and drying it in an oven at 90 °C until a constant weight was reached. Labelled OPFR standards were added prior to analysis by turbulent flow chromatography (TFC) coupled to LC-MS/MS.

2.4. Instrumental analysis

Purification was performed on-line at the beginning of the instrumental analysis with a Thermo Scientific TurboFlow™ system (Giulivo et al., 2016). Columns used were CycloneTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm) in combination for purification. An analytical column (Purosphere Star RP-18, 125 mm × 0.2 mm) was used for chromatographic separation. The mobile phase was a gradient of water (0.1% formic acid) and methanol (ammonium acetate) at 0.75 mL min^{-1} (Santín et al., 2016). Mass spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source. For all compounds, selective reaction monitoring (SRM) mode was used with two transitions.

2.5. Quality assurance

A blank was included every 10 samples. If the blank signal did not exceed 10% of sample signals, OPFR levels of the blank were subtracted from the corresponding batch of samples. If the blank signal was higher, samples were re-analysed. All the non-volumetric material was heated at 340 °C and rinsed with the appropriate solvent before use, and no plastic material was used to avoid contamination. Recoveries were 48–102%, with RSDs between 0.29 and 24.7%. Limits of detection (LODs) and limits of quantification (LOQs) were 0.19–19.3 ng g^{-1} lw and 0.97–24.8 ng g^{-1} lw, respectively.

2.6. Expression of concentrations

OPFRs are lipophilic compounds and, as such, their concentrations are usually recommended to be expressed on a lw basis to control for varying lipid content between species, individuals and tissues (Krahn et al., 2003). However, here we detail the concentrations of OPFRs expressed on three bases: extractable lipid basis (lw), fresh weight basis (fw), and dry weight basis (dw) to allow comparison with previous studies. Irrespective of this, when the evaluation of concentrations involves different tissues, the data used are always those expressed on a lw basis; if they are not given in the original source, conversion from other bases is made using relevant data on tissue lipid content available in the literature.

2.7. Statistical analysis

For statistical analyses, the whales were divided into three growth stages: sexually immature whales, sexually mature females and sexually mature males. This grouping was done because the three groups usually present different burdens of lipophilic pollutants. In young whales the pollutant concentrations in specimens from the two sexes are usually indistinguishable. In females, pollutants tend to decrease due to the transfer to offspring which occurs during pregnancy and lactation. Obviously, the transfer begins at the onset of reproductive activity and, because of this, mature females tend to have lower levels than males and juveniles (Aguilar and Borrell, 1988). The normality and heteroscedasticity of the distribution of ΣOPFR (the sum of all OPFR compounds) concentrations in fin whales muscle and in krill were preliminary tested using the Shapiro Wilk and Levene's tests, respectively. Whenever the tests showed that data distribution departed from normality, comparisons were made using the non-parametric Kruskall-Wallis rank sum test. The significance level was set at $p < .05$. Calculations were carried out with the programming environment R (R Core Team, 2018).

3. Results

ΣOPFR concentration per individual whale is shown in Table 1. Mean concentrations of the single OPFR compounds in fin whales and in krill are summarized in Table 2. OPFRs were detected in all samples, and at concentrations ranging between 31.9 and 10,232 ng g^{-1} lw in fin whales (Table 1), and between 74.8 and 3764 ng g^{-1} lw in krill. Out of 19 OPFR compounds, 7 were detected in the fin whale samples and 5 in the krill samples. IPPP, TNBP and TPPO were the most abundant compounds in both species (Fig. 1) and they were detected, respectively, in 10, 65 and 55% of fin whale samples and in 50, 70 and 50% of krill samples. 4IPPDPP was the most frequent OPFR in fin whales, being detected in 90% of samples, while 4IPPDPP and 2IPPDPP were the most frequent OPFRs in krill, being detected in 90% and 100% of samples, respectively. TPP and DCP were only detected in fin whales and showed a frequency of detection of 35 and 5%, respectively.

ΣOPFR concentrations had homogeneous variances in fin whales and krill ($p = .63$, Levene test), and followed a normal distribution ($p = .15$, Shapiro Wilk test) in mature fin whales, while they did not follow a normal distribution in immature fin whales and krill ($p < .05$, Shapiro Wilk test). Taking this into account, ΣOPFR concentrations between fin whale sexes and reproductive status, and between fin whales and krill, were compared using the non-parametric Kruskal-Wallis rank sum test. No differences were detected in ∑OPFR concentrations in fin whales between immatures, mature females and mature males (1546 ± 3117; 319 ± 214 and 470 ± 462 ng g^{-1} lw, respectively; $p = .90$, Kruskall-Wallis rank sum test; Fig. 2). Similarly, no differences were detected in ∑OPFR concentrations between fin whale and krill (985 ± 2238 and 949 ± 1090 ng g^{-1} lw, respectively; $p = .29$, Kruskall-Wallis rank sum test; Table 2).

4. Discussion

In this study, the presence and concentrations of plasticizers and flame retardants of the OPFR family were investigated in North Atlantic fin whales and their main prey, the krill *Meganyctiphanes norvegica*. Three main results were obtained: 1) OPFRs are present both in North Atlantic fin whales and in krill, 2) OPFRs did neither bioaccumulate nor biomagnify in fin whales and their prey, and 3) considering both the concentration and the frequency of detection, TNBP was the main OPFR detected in both species.

Table 2
Individual OPFR-compound concentrations expressed in ng g^{-1} ww, dw and lw (mean ± SD) in fin whale muscle and krill samples collected off Iceland.

	Fin whale			Krill		
	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})
TPPO	1.84 ± 1.72	7.67 ± 7.15	128 ± 189	1.35 ± 1.48	7.52 ± 7.93	114 ± 155
TPP	0.74 ± 1.13	2.82 ± 4.17	59.2 ± 94.9	nd[a]	nd[a]	nd[a]
TNBP	3.21 ± 2.59	13.1 ± 10.2	214 ± 281	2.71 ± 2.60	15.7 ± 12.6	165 ± 158
DCP	0.29 ± 1.30	1.17 ± 5.25	8.79 ± 39.3	nd[a]	nd[a]	nd[a]
2IPPDPP	0.41 ± 0.69	1.77 ± 2.95	39.6 ± 75.0	1.75 ± 1.17	9.92 ± 7.35	116 ± 108
4IPPDPP	0.83 ± 0.48	3.40 ± 1.85	69.4 ± 61.8	1.42 ± 0.82	7.80 ± 4.62	97.6 ± 107
IPPP	5.89 ± 26.3	24.0 ± 107	465 ± 2076	5.39 ± 8.12	31.1 ± 43.8	457 ± 792
ΣOPFRs	13.2 ± 27.8	54.0 ± 113	985 ± 2238	12.6 ± 9.46	72.1 ± 50.6	949 ± 1090
Range	0.51–129	2.24–528	31.9–10,232	2.66–36.2	13.0–186	74.8–3764

[a] nd: non detected (below detection limits).

4.1. OPFR concentrations

In the present study we report the first evidence of OPFR presence in fin whales and in their main prey. Data about OPFRs in marine mammals is scarce: to our knowledge, only 6 studies have been published; two of them on polar bears and phocids, and four on odontocetes, but none on a mysticete as the fin whale is.

The specific OPFR compounds detected in the plasma of polar bears (*Ursus maritimus*) and phocids from Norway by Hallanger et al. (2015) were different from those found in the current study, although in both studies were analysed TCEP, TCIPP, TBOEP, TEHP, TPHP, EHDPP and TNBP. In another study done in polar bears and phocids from Greenland (Strobel et al., 2018), TNBP was found to be the highest and most frequent compound, a result consistent with the present study, in which TNBP ranked as second compound with highest concentrations. However, a direct comparison of these results with those from the present study cannot be made because concentrations are expressed on a ww basis, so we recalculated the data using a value of 89.3% ($n = 92$) for Greenland polar bears (Dietz et al., 2007) and 93% ($n = 100$) for Greenland ringed seals (Cleemann et al., 2000). Resulting concentrations on a lw basis were 0.87 ng g^{-1} and 1.4 ng g^{-1}, respectively, far lower than those found in the current study, suggesting that Greenland would be less affected by plastic pollution than Iceland.

The first report on OPFRs in odontocetes was that by Papachlimitzou et al. (2015), who analysed flame retardants and plasticizers in the liver and blubber of harbour porpoises (*Phocoena phocoena*) from the UK. These authors detected 6 OPFR compounds out of the 20 analysed and found TPPO in 11 out of 19 blubber samples. Again, we had to transform the original values, expressed on a ww basis, into lw basis assuming a mean lipid content in blubber of 85%, as reported from individuals from the same species and population (Law et al., 2010). The converted results indicate that TPPO concentrations were more than three times lower in the blubber of porpoises (38 ng g^{1} lw) than in the muscle of fin whales (128 ng g^{1} lw). Putting aside potential differences in the analytical procedures between laboratories, the difference is large enough and it may be taken as an indication that, in the North Atlantic, fin whales are more contaminated by OPFRs than porpoises. However, we should consider this conclusion with caution because the comparison involves different tissues and our present understanding on the dynamics and compartmentation of OPFRs in the body of mammals is poor.

Two recent studies on OPFRs in cetaceans have focused on Mediterranean dolphins. Sala et al. (2019) detected 12 out of the 16 OPFR compounds analysed in the muscle, liver, kidney, blubber and brain of common dolphins (*Delphinus delphis*) from the Alboran Sea. They found, among other compounds, TNBP, TPPO, DCP and IPPP, consistently with what we found in fin whales. Mean ∑OPFRs in dolphin muscle was similar to the values we found in the fin whale muscle (994 and 985 ng g^{-1} lw, respectively). Also, Aznar-Alemany et al. (2019) analysed OPFR compounds in three dolphin species (*Delphinus capensis*, *Sousa plumbea* and *Tursiops aduncus*) from the Indian Ocean, and, consistently with our results, they detected TNBP, TPPO, and IPPP. In this case, ∑OPFR concentrations in the muscle of the various dolphin species ranged between 1630 and 31,861 ng g^{-1} lw, with a mean value of 10,452 ng g^{-1} lw. These concentrations are one order of magnitude higher than those observed in the present study and in the Mediterranean dolphins, this time more clearly supporting that pollution by plastic derivatives is higher in the Indian Ocean than in the Atlantic Ocean.

If information on OPFR concentrations in marine mammals is scarce, data about OPFRs in plankton is even scarcer: Zhao et al. (2018) analysed OPFR levels in plankton and crustaceans from the Taihu lake, in China, detecting 13 out of the 14 OPFRs analysed. In consistency with our results, authors also reported TNBP to be one of the major contributors to the ∑OPFR, although mean TNBP concentration in North

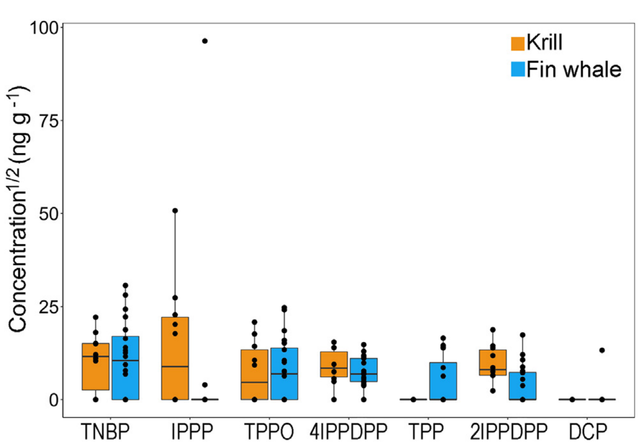

Fig. 1. Concentrations of OPFR compounds (lipid weight basis) detected in fin whales and krill off Iceland. OPFR concentrations were normalized using the square root.

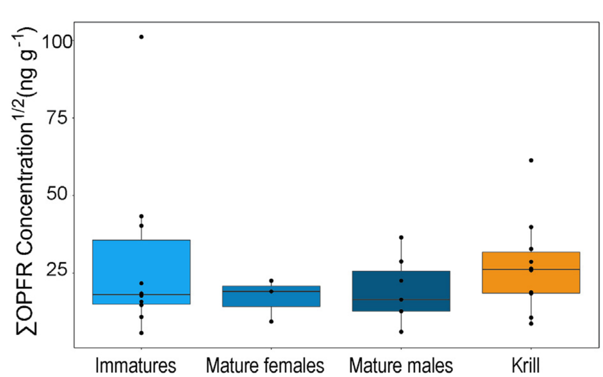

Fig. 2. Box-plots illustrating the ∑OPFR concentrations in fin whales grouped by immatures, mature females and mature males, and ∑OPFR concentrations in krill. ΣOPFR concentrations were normalized using the square root.

Atlantic krill (2.71 ng g^{-1} ww) was 5 times higher than the concentrations reported by Zhao et al. (2018) (0.54 ng g^{-1} ww).

The information on potential health effects caused by TPPO, TPP, IPPP, 2IPPDPP and 4IPPDPP, which are commonly used as flame retardants and plasticizers, is insufficient. Conversely, toxic effects of DCP and TNBP are better studied. In rats, mice, rabbits and guinea pigs, the DCP median lethal dose (LD_{50}) has been reported to be higher than 1000 ng g^{-1} (US-EPA, 2007; van der Veen and de Boer, 2012), which is three orders of magnitude higher than the concentrations found in the tissues of fin whales. TNBP toxicity in biota is also well established (van der Veen and de Boer, 2012), with LD_{50} ranging from 1400 to 3200 ng g^{-1} in different animals (Bruchajzer et al., 2015). In rats, TNBP may produce organ damages (kidneys, liver and urinary bladder epithelium), neurotoxic effects, cancer of urinary bladder and impairment of fertility (Arnold et al., 1997; Auletta et al., 1998a; Auletta et al., 1998b; Bergman et al., 2012; Bruchajzer et al., 2015; Tyl et al., 1997). TNBP concentrations found in the muscle of fin whales ranged from nd to 6.56 ng g^{-1} ww: values three orders of magnitude lower than those referred to as LD_{50} in laboratory animals.

From this, we can conclude that the potential toxic impact of OPFRs on fin whales is limited. However, OPFR concentrations are likely to in-crease in the marine environment and in marine organisms as pollution from plastic marine litter increases in seas and oceans worldwide. As seen, the fin whale, similarly to other filter-feeding and long-range migrating mysticetes, stands as a collective of species potentially suscepti-ble to be severely affected by these emerging contaminants. Moreover, their long life span (~80–90 years, Aguilar and García-Vernet, 2018), would extend fin whales exposure to these pollutants over very long periods of time. Thus, these species should be monitored with particular attention, and the effects of OPFRs must be assessed on a medium –large temporal scale, as they may lead to chronic toxicity.

4.2. Bioaccumulation and biomagnification of OPFRs

Although a few studies in biota have suggested that OPFRs such as TNBP or DCP may bioaccumulate, this is, increase with lifespan (Giulivo et al., 2017), others show that they can be readily metabolised and that, as a result, their concentrations do not build-up with age (Greaves et al., 2016; Regnery and Püttmann, 2010; Strobel et al., 2018; Van den Eede et al., 2013). In this latter line of findings, Aznar-Alemany et al. (2019) did not observe differences in $\sum$OPFR concentra-tions between juveniles and adult male dolphins. Consistently, our re-sults on fin whales did not show significant variation in $\sum$OPFR concentrations between immature individuals and reproductive mature males or females, and thus did not suggest the occurrence of bioaccu-mulation processes through lifespan, consistently with what happens in other mammals (Aznar-Alemany et al., 2019).

Mean $\sum$OPFR concentrations were similar in mature females and mature males, although they were slightly higher in males (470 $\pm$ 462 and 319 $\pm$ 214 ng g^{-1} lw, respectively). In several mammals, including cetaceans, organochlorine and other lipophilic pollutants, such as PCB or DDT, are transferred from mothers to their offspring during gestation and lactation (Aguilar and Borrell, 1994; Borrell and Aguilar, 2005; Borrell et al., 1995). Therefore, the concentration of these pollutants in mature females is usually lower than that in mature males. Along with the age-related decline in pollutant body loads in females, the transfer rates during reproduction tend to decrease with the mother's age, all wich results in the first calf delivered being the one receiving the largest pollutant loads (Aguilar and Borrell, 1994; Borrell et al., 1995). The current results, with an absence of significant differences in OPFR tissue concentrations between mature males and mature females, appear to indicate that any reproductive transfer is occurring, or that if it does, is very limited. However, because mother-calf transfer of OPFRs has not been assessed in any mammal species and, given the potential parallelism in the dynamics of OPFRs with that of other lipophilic pollutants, its assessment and potential toxicological implications should be a priority for future research.

Evidences for biomagnification, this is, the building-up of $\sum$OPFR concentrations through the food web, are limited and conform an unclear picture (Du et al., 2019). Hallanger et al. (2015) found weak biomagnification of OPFRs from fish to their predators, and Strobel et al. (2018) also found limited OPFR biomagnification from ringed seals to polar bears. However, Brandsma et al. (2015) and Zhao et al. (2018) analysed the trophic transfer through entire marine food webs and found that the majority of OPFRs exhibit trophic dilution and thus do not biomagnify or even follow negative relationships between concentration and aquatic species trophic level. One likely explanation for these latter results may be the rapid metabolism of OPFRs into other compounds (Hou et al., 2016; Strobel et al., 2018; Zhao et al., 2018). Consistently, the fact that in our study the mean $\sum$OPFR concentrations did not significantly differ between the fin whale muscle and krill, seems to indicate that biomagnification through the food web is limited for these compounds.

Of the 7 OPFR compounds detected in fin whale, 5 were also detected in krill samples, suggesting that the OPFR intake in fin whales is mainly derived from the diet. However, DCP and TPP were detected in fin whale muscle but not in krill samples, indicating a different source of uptake for these compounds. A possible explanation is the direct intake of the chemical additives (*i.e.*, TPP and DCP) from the ingested micro- or macro-plastic in the fin whale stomach, and subsequent transfer to the whale tissues. Although no studies have been specifically conducted in fin whales from Icelandic waters, others carried out in other areas (*i.e.*, Besseling et al., 2015; Fossi et al., 2014; Baulch and Perry, 2014) have reported the presence of plastics in the gastrointestinal tract of baleen whales, reinforcing this argument. Similarly, other pollutants, such as higher-brominated diphenyl ethers (Tanaka et al., 2013) and phthalates (Hardesty et al., 2015) have been detected in seabirds but not in their prey, suggesting a direct intake of these compounds from ingested plastics.

2IPPDPP and IPPP were found in both the fin whales and the krill samples, but they were detected in a smaller proportion in the former (40% and 10%, respectively) than in the latter (100% and 50%, respectively). These results may be related to a more efficient metabolism and excretion of these compounds in fin whales than in krill. In addition, IPPP was only found in immature fin whales, suggesting that the capacity of metabolising this compound is acquired during lifespan. An alternative explanation may be the partial contribution of ingested plastics to the overall pollutant load built by krill ingestion. Focused research is needed to clarify this hypothesis, to better understand the processes involved in the metabolism of OPFRs in fin whales and the mechanisms through which these compounds are transferred to the whale tissues and at what magnitude.

5. Conclusions

Results of this paper provide the first evidence of OPFR presence in the muscle of North Atlantic fin whales and in their main prey, the krill *Meganyctiphanes norvegica*, with TNBP being the most concentrated OPFR in both organisms. OPFR bioaccumulation in fin whales and biomagnification from krill to whales were not observed. Our results remark the potential of fin whales to be used as biomonitors of OPFR pollution at large-scale, due to their wide distribution, high potential susceptibility to plastic-related pollutants as filter-feeding animals, and high mobility. Further research is needed to assess the potential toxic effects of TNBP to fin whales and krill, and to investigate the processes of OPFR intake, metabolism, reproductive transfer and overall dynamics in long-lived, filter-feeding cetaceans performing long-range migrations, such as fin whales.

CRediT authorship contribution statement

Odei Garcia-Garin: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Berta Sala:** Methodology, Validation, Supervision. **Alex Aguilar:** Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Morgana Vighi:** Writing - original draft, Writing - review & editing, Supervision. **Gísli A. Víkingsson:** Resources. **Valerie Chosson:** Resources, Writing - original draft. **Ethel Eljarrat:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Asunción Borrell:** Conceptualization, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

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3.3. Intrapopulation and temporal differences of phthalate concentrations in North Atlantic fin whales *(Balaenoptera physalus)*

Autores: Odei Garcia-Garin[1*], Wissam Sahyoun[2], Sopheak Net[2], Morgana Vighi[1], Alex Aguilar[1], Baghdad Ouddane[2], Gísli A. Víkingsson[3], Valerie Chosson[3], Asunción Borrell[1]

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

[2]Université de Lille 1, Sciences et Technologies, Laboratoire LASIR (UMR 8516 CNRS), Cité Scientifique, 59655, Villeneuve d'Ascq, France

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

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Abstract:
The fin whale (*Balaenoptera physalus*) is a migratory filter-feeding species that is susceptible to ingest plastics while lunge feeding across the oceans. Plastic additives, such as phthalates, are compounds that are added to plastics to give them specific characteristics, such as flexibility. These so-called plasticizers are currently raising major concern because of their potential adverse effects on marine fauna. However, little is known about phthalate concentrations in tissues of baleen whales as well as their potential relation with biological variables (*i. e.,* sex, body length and age) and their trends with time. In this study, we assessed the concentration of 13 phthalates in the muscle of 31 fin whales sampled in the feeding grounds off western Iceland between 1986 and 2015. We detected 5 of the 13 phthalates investigated, with di-*n*-butylphthalate (DBP), diethylphthalate (DEP) and bis(2-ethylhexyl) phthalate (DEHP) being the most abundant. None of the biological variables examined showed a statistically significant relationship with phthalate concentrations. Also, phthalate concentrations did not significantly vary over the 29-year period studied, a surprising result given the global scenario of increasing plastic pollution in the seas. The lack of time trends in phthalate concentration may be due in part to the fact that phthalates also originate from other sources. Although no adverse effects of phthalates on fin whales have been detected to date, further monitoring of these pollutants is required to identify potential toxic effects in the future.

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Intrapopulation and temporal differences of phthalate concentrations in North Atlantic fin whales (*Balaenoptera physalus*)

Odei Garcia-Garin [a,*], Wissam Sahyoun [b], Sopheak Net [b], Morgana Vighi [a], Alex Aguilar [a], Baghdad Ouddane [b], Gísli A. Víkingsson [c], Valerie Chosson [c], Asunción Borrell [a]

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

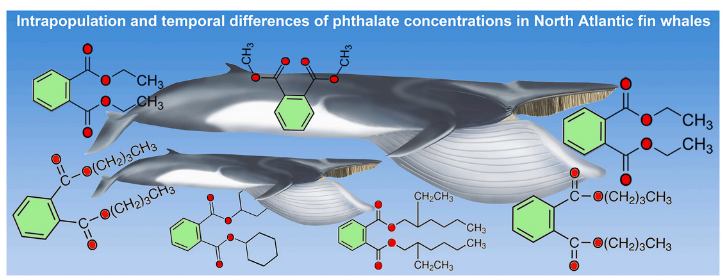
[b] *Université de Lille 1, Sciences et Technologies, Laboratoire LASIR (UMR 8516 CNRS), Cité Scientifique, 59655, Villeneuve d'Ascq, France*

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

HIGHLIGHTS

- Out of 13 phthalate compounds investigated, 5 were detected.
- DBP, DEP and DEHP were the most abundant forms.
- Phthalate concentrations were not significantly different between sexes.
- Phthalates concentrations were not significantly different between age classes.
- Phthalate concentrations did not show temporal differences.

GRAPHICAL ABSTRACT



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ABSTRACT

The fin whale (*Balaenoptera physalus*) is a migratory filter-feeding species that is susceptible to ingest plastics while lunge feeding across the oceans. Plastic additives, such as phthalates, are compounds that are added to plastics to give them specific characteristics, such as flexibility. These so-called plasticizers are currently raising major concern because of their potential adverse effects on marine fauna. However, little is known about phthalate concentrations in tissues of baleen whales as well as their potential relation with biological variables (*i.e.,* sex, body length and age) and their trends with time. In this study, we assessed the concentration of 13 phthalates in the muscle of 31 fin whales sampled in the feeding grounds off western Iceland between 1986 and 2015. We detected 5 of the 13 phthalates investigated, with di-*n*-butylphthalate (DBP), diethylphthalate (DEP) and bis(2-ethylhexyl) phthalate (DEHP) being the most abundant. None of the biological variables examined showed a statistically significant relationship with phthalate concentrations. Also, phthalate concentrations did not significantly vary over the 29-year period studied, a surprising result given the global scenario of increasing plastic pollution in the seas. The lack of time trends in phthalate concentration may be due in part to the fact that phthalates also originate from other sources. Although no adverse effects of phthalates on fin whales have been detected to date, further monitoring of these pollutants is required to identify potential toxic effects in the future.

* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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1. Introduction

Phthalates are broadly used as additives in plastics to give them flexibility and transparency, similarly as in other consumer products, like cosmetics or perfumes (Hansen et al., 2013). They have become ubiquitous environmental chemicals (Net et al., 2015) and, as such, they are frequently detected. Indeed, they have been reported to occur in several different matrices including air (*e.g.*, Hwang et al., 2008), soil (e.g., Zeng et al., 2009), sediments (*e.g.*, Blair et al., 2009), fresh and sea waters (*e.g.*, González-Mariño et al., 2017; Huang et al., 2008; Xie et al., 2007) and the tissues of marine biota (*e.g.*, Güven and Coban, 2013; Routti et al., 2021). It has been hypothesized that despite the rapid metabolism and elimination of most phthalates (Bang et al., 2011), stable tissue concentrations may be maintained through chronic low-level exposure through dietary ingestion (Silva et al., 2004). Due to these properties and their potential toxicity, phthalates are of special concern.

Studies on the adverse health effects of phthalates in laboratory animals (rats and mice), as well as epidemiological studies conducted in humans, indicate that phthalates act as hormone sensitizers and nuclear receptors (Baken et al., 2019; Benjamin et al., 2017; NRC, 2008). Some of these compounds, such as bis(2-ethylhexyl) phthalate (DEHP) and di-*n*-butylphthalate (DBP), appear to impair reproduction, metabolism and development, and to cause neurological and carcinogenic effects (Benjamin et al., 2017; NRC, 2008). Furthermore, in vitro studies showed that DEHP transactivates the thyroid hormone receptor of fin whales (*Balaenoptera physalus*) (Routti et al., 2021). However, the actual impact of these compound on wild animals is yet to be further assessed.

Pollution by marine litter is increasing in all marine basins worldwide (*e.g.*, Lebreton et al., 2018). Since more than 80% of marine litter is composed of plastics (*e.g.*, Garcia-Garin et al., 2020a,b; UNEP/MAP, 2015), and plastics contain between 10 and 60% of phthalates (Fromme, 2011), marine litter can be a major source of phthalate pollution in the sea. Moreover, the release and dispersion of phthalates in the marine environment is facilitated by the fact that these compounds are not chemically bound to plastics (Hahladakis et al., 2018). On the other hand, atmospheric transport is also a relevant pathway for long-range transport and eventual deposition of phthalates in the marine environment (Xie et al., 2007).

However, information on phthalate concentrations in the marine fauna, and especially in the tissues of marine mammals, is scarce. To our best knowledge, fifteen species of cetaceans in ten scientific articles have been investigated on this topic until now, namely: fin whale (Baini et al., 2017; Fossi et al., 2012, 2014, 2016; Routti et al., 2021), bottlenose dolphin (*Tursiops truncatus*) (Baini et al., 2017; Dziobak et al., 2021; Hart et al., 2018, 2020; Montoro-Martínez et al., 2021; Page-Karjian et al., 2020), harbour porpoise (*Phocoena phocoena*) (Rian et al., 2020), Fraser's dolphin (*Lagenodelphis hosei*), Risso's dolphin (*Grampus griseus*), and short-finned pilot whale (*Globicephala macrorhynchus*) (Montoro-Martínez et al., 2021), pygmy sperm whale (*Kogia breviceps*) (Montoro-Martínez et al., 2021; Page-Karjian et al., 2020), striped dolphin (*Stenella coeruleoalba*) (Baini et al., 2017; Montoro-Martínez et al., 2021), melon-headed whale (*Peponocephala electra*), Blainville's beaked whale (*Mesoplodon densirostris*), dwarf sperm whale (*Kogia sima*), pantropical spotted dolphin (*Stenella attenuata*), white-beaked dolphin (*Lagenorhynchus albirostris*), and Atlantic spotted dolphin (*Stenella frontalis*) (Page-Karjian et al., 2020), blue whale (*Balaenoptera musculus*) and bowhead whale (*Balaena mysticetus*) (Routti et al., 2021). The dynamics of these pollutants as related to the species biological variables remains to be clarified.

Baleen whales are filter-feeding, long-lived species that carry out long-range migrations (*e.g.*, Aguilar and García-Vernet, 2018). During lunge feeding, they are liable of ingesting floating plastics (Garcia-Garin et al., 2020c, 2021), and thus they are considered potentially good indicator species to assess the occurrence of microplastics (*e.g.*, Garcia-Garin et al., 2021) or toxic compounds related to plastic pollution, such as organophosphate esters (Garcia-Garin et al., 2020c), in large water masses. Indeed, baleen whales have been previously used to monitor the chemo-physical characteristics of oceanic water masses (Borrell et al., 2018).

In the current study we investigated phthalate concentrations in the muscle of North Atlantic fin whales (*Balaenoptera physalus*) sampled off Iceland along a period of thirty years (1986–2015) with a twofold objective: i) to examine any potential relation between phthalate concentration in muscle and the main biological variables of the sampled individuals (age, sex and body length), and ii) to investigate temporal differences in the concentrations of phthalates in the analysed tissue.

2. Materials and methods

2.1. Study area and sampling

Muscle samples were collected from 31 fin whales (14 males, 17 females) caught off western Iceland and flensed at the Hvalur H/F whaling company (Fig. 1) during the summers of 1986, 2009, 2013 and 2015. The body length of these whales ranged from 16.8 to 20.6 m and their age ranged from 7 to 47 years old (Table 1). Stainless steel material was used to cut through the muscle tissue and manipulate the samples, of which about 40 g per sampled whale were collected and placed in glass bottles. Field blanks were not made, as weather and factory conditions did not facilitate this procedure. To avoid contamination, no plastic material was used. After collection, samples were stored at −20 °C until analysis.

2.2. Ethics statement

Samples were obtained from whales legally caught under Icelandic regulation and were legally imported in Spain. Export/import licenses were obtained from the respective Icelandic and Spanish authorities and the samples transported under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Export/import permit numbers are: 13ISO28MA and 15IS017MA/ESBB00222/13I and ESBB00207/15I, respectively. No samples were donated or purchased and all sampled whales were caught with purposes other than research.

2.3. Age determination

Age determination was performed at the Marine and Freshwater Research Institute according to methods described by Lockyer (1984). Growth layers groups (GLG) were counted through a longitudinal section of the ear plug core and each pair of GLGs was assumed to

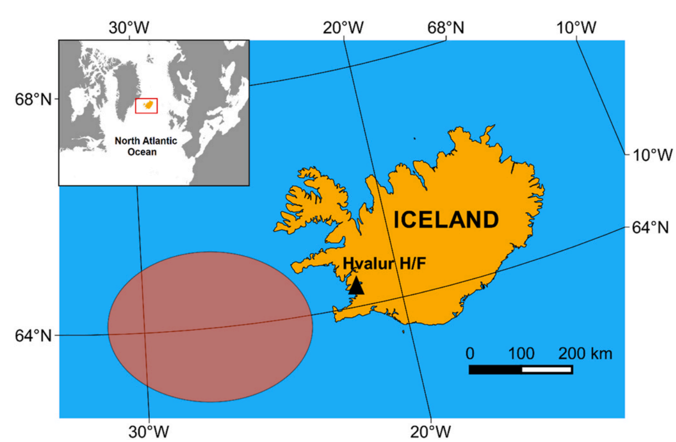

Fig. 1. Area of fin whales catches (red ellipse) and of the whaling factory where fin whales (n = 31) were processed (black triangle). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Biological traits and ∑phthalate concentrations in muscle (expressed in ug g^{-1} lipid weight (lw)) for each fin whale (n = 31) collected off western Iceland. Non detected compounds were computed as 0.

ID	Year	Sex	Length (m)	Age (years)	ΣPAE (μg g^{-1} lw)
1486	1986	F	18.6	16	8.982
1986	1986	M	20.1	23	5.562
2586	1986	F	20.4	27	8.315
3886	1986	M	17.4	14	1.186
4186	1986	F	19.5	35	0.546
4286	1986	F	20.1	17	0.245
4986	1986	F	20.1	26	6.690
F09013	2009	M	17.7	31	2.903
F09022A	2009	F	20.1	17	6.015
F0906	2009	F	17.0	7	2.445
F09093	2009	M	18.2	47	15.058
F09095	2009	F	18.6	21	5.176
F09104	2009	M	19.2	29	5.705
F09112	2009	M	17.8	32	2.656
F09117	2009	F	19.3	23	5.263
F0980	2009	F	16.8	10	34.415
F13002	2013	F	20.0	26	5.384
F13005	2013	F	20.6	28.5	5.574
F13018	2013	F	19.1	24.5	1.301
F13030	2013	M	18.3	15.5	19.289
F13059	2013	M	17.6	25	9.681
F13075	2013	F	18.9	41	6.460
F13099	2013	M	19.0	23	11.018
F15007	2015	M	18.3	30	1.470
F15013	2015	F	20.4	–	13.603
F15017	2015	M	18.0	–	6.089
F15031	2015	M	17.4	–	17.401
F15059	2015	F	19.5	–	37.514
F15071	2015	M	17.1	36	6.332
F15088	2015	M	17.1	32	7.078
F15146	2015	F	20.4	41	4.345
Mean ± SD					8.506 ± 8.706

correspond to one year. Each count was repeated by more than one reader, and for about 70% of samples the count was repeated twice by the same reader. The age of four individuals was not reported because GLGs were not optimal for age determination (Table 1).

2.4. Standards and reagents

A total of thirteen phthalates (Table S1) were analysed, namely: dimethylphthalate (DMP), diethylphthalate (DEP), di-*n*-butylphthalate (DBP), bis(2-methoxyethyl) phthalate (DMEP), dipentylphthalate (DPP), bis(2-ethoxyethyl) phthalate (DEEP), benzyl butyl phthalate (BBP), phthalic acid dicyclohexyl ester (DCHP), bis(4-methyl-2-pentyl) phthalate (BMPP), di-*n*-hexyl phthalate (DHP), bis(2-*n*-butoxyethyl) phthalate (DBEP), bis(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DNOP). Analytical standards were purchased from Restek (Lisses, France). Labelled phthalic acid diisobutyl ester (d_4-DIBP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and ethyl acetate (for HPLC isocratic grade) for trace analysis were purchased from VWR Chemicals BDH. Acetone (SupraSolv®) was sourced from Merck. The extraction salts were purchased from Agilent Technologies.

2.5. Sample preparation

Samples of approximately 20 g of frozen muscle were lyophilised for 48 h. Then, a subsample of 4 g dry weight (dw) was taken from the central part of each sample (to avoid any contamination) and homogenised. Subsequently, 10 mL of acetonitrile, 10 mL Milli-Q water, 100 μL of internal standard (d_4-DIBP) and the extraction salts (1 g of NaCl, 4 g of $MgSO_4$, 1 g of $C_6H_5Na_3O_7{\cdot}2H_2O$ and 0.5 g of $C_6H_6Na_2O_7{\cdot}1.5H_2O$) were added to the sample in a 50 mL centrifuge tube. The mixture was stirred with ceramic homogenizers in a vortex mixer and centrifuged during 5 min at 5000 rpm. Subsequently, 8 mL of extract were transferred to another centrifuge tube containing the salts (400 mg PSA, 400 mg GCB, 1200 mg MgSO4 and 400 mg C18EC) for the dispersive solid phase extraction clean-up step. After vortexing and centrifugation during 5 min at 5000 rpm, 5 mL of supernatant were transferred to a vial. The extraction was carried out twice, the second time using 10 mL of ethyl acetate. Both extracts were combined, reduced under a gentle nitrogen stream to 200 μL, and stored in a glass tube in the freezer until GC-MS/MS analysis was performed.

Lipid weight (lw) was determined gravimetrically from 1 g dw of each sample by sonication using 15 mL of a solution hexane:acetone (1:1) during 15 min, following Garcia-Garin et al. (2020c). The extraction was carried out twice, and both extracts were combined. Then, the solvent was evaporated using a nitrogen stream and the remaining lipids dried at 90 °C until a constant weight was reached.

2.6. Instrumental analysis

All analyses were performed using an Agilent 7890 GC coupled to an Agilent 7000C Triple Quadrupole GC/MS system (Agilent Technologies, France). To provide analyte separation an Agilent DB-17 ms, 30 m × 0.25 mm, 0.25 μm column was used, with helium (99.999% purity) as carrier gas at a constant flow of 1.2 mL/min.

Sample injections were performed in a multimode inlet, operated using the solvent vent injection mode through an ultra-inert inlet liner, with a glass wool frit from Agilent.

The injector operating conditions were as follows: injection volume was 3 μL; the injector temperature was held at 50 °C during the solvent evaporation stage (0.8 min), ramped up to 300 °C at 720 °C/min (hold 5 min), and cooled down to 280 °C (hold 10 min). Helium (99.999% purity) at a flow rate of 2.25 mL/min was used as the quenching gas; and nitrogen (99.999% purity) at a flow rate of 1.5 mL/min as the collision gas. The oven temperature was set as follows: 40 °C for 2 min, programmed to 220 °C at 30 °C/min, then to 260 °C at 5 °C/min and finally to 280 °C at 20 °C/min (hold 7 min). The total run time was 24 min. The triple quadrupole mass spectrometer was operated in electron impact ionisation (EI) with an ionising energy of 70 eV, and in the MRM mode. The temperatures of the transfer line, ion source and quadrupole 1 and 2 were 280 °C, 230 °C and 150 °C, respectively.

2.7. Quality assurance

A blank was included in the analytical run series every 10 samples. If the blank signal did not exceed 25% of sample signals (which was the case for all blanks), the phthalate concentration of the blank was subtracted from the corresponding batch of samples (Net et al., 2015). The procedure was performed in clean laboratory conditions, using glass or metal equipment instead of plastic (both in the field and in the laboratory). All glassware was rinsed with the appropriate solvent (water + detergent, Milli-Q water + HCl, Milli-Q water, and acetone) before use. Sample and standard preparation, as well as extraction and clean-up, were performed in a laminar flow cabinet. Good calibration curves were obtained in the range of 0.1–5000 μg L^{-1} with the correlation coefficients of ≥0.994. Phthalates were non-detected in the solvents. Limits of quantification (LOQs) were determined for each compound based on the average background noise or the concentration in the procedural blanks plus ten times the standard deviation and were verified by the analysis. LOQs were in the range of 0.025–0.125 μg L^{-1} of the extract which correspond to the range of 0.012–0.062 ng g^{-1} dw. The recovery of each phthalate was calculated by spiking the targeted compounds into real matrix of interest. The mean recovery was 92% with the rate of 104, 81, 86, 98 and 76 for DMP, DEP, DBP, DEHP and DCHP, respectively. The repeatability of the analysis procedure, the standard deviations were obtained <5% when the concentration of targeted compound higher than 0.1 ng g^{-1} and ~12% when the concentration of each phthalate was close to LOQs.

All analyses were performed at the LASIRE laboratory (UMR CNRS 8516) of the University of Lille, France.

2.8. Expression of concentrations

As phthalates are lipophilic compounds, it is usually recommended to report their concentrations on a lw basis to compensate for varying lipid content between tissues, individuals and species (Krahn et al., 2003). However, to allow comparison with other studies, here we report the concentrations of phthalates expressed both on an extractable lipid basis (lw) and on a dry weight basis (dw).

2.9. Statistical analysis

The normality and heteroscedasticity of the distributions of phthalate concentrations were preliminarily tested using the Shapiro Wilk and Barlett tests, respectively. As data distribution departed from normality, it was normalized applying a square root transformation. Sex, age and length distributions showed no difference between the two year groups (1986 and 2009–2015) (p-value < 0.05, Kruskal-Wallis rank sum test). PERMANOVAs (Oksanen et al., 2020) were used to explore the potential effect of "year" (*i.e.*, year of sample collection; expressed as a fixed factor: 1986 or 2009–2015), "sex" (*i.e.*, male or female), "length" and "age" on the concentration of phthalates. PERMANOVA equations can be found in Anderson (2001). "Length" and "age" were not correlated (p-value $= 0.42$; Pearson's correlation test). To allow the inclusion of individuals of unknown age in the analysis, additional PERMANOVAs were created by excluding the "age" factor and fitted with "year", "sex" and "length". The most complex model, which included the factors "year", "sex", "length" and "age" (including the biologically relevant interactions), was subjected to sequential, stepwise simplification by deleting one term (whether it was an interaction or a main effect). The information-theoretic approach was used for model selection (Burnham and Anderson, 2002) and models were compared using the AIC (Akaike's Information Criterion) (Akaike, 1974) and the deviance explained. The level of significance was set at $p < 0.05$. Analyses were conducted using the vegan package (Oksanen et al., 2020) in R (R Core Team, 2021).

3. Results

Total phthalate concentrations (*i.e.*, the sum of the single compound concentrations) for each whale are shown in Table 1. At least one phthalate compound was above the limit of quantification in every sample and the total phthalate concentration, in the whales, ranged from 0.245 to 37.514 μg g^{-1} lw (Table 1). The median concentration of each phthalate compound, from all the tested whales, is summarized in Table 2. Out of 13 phthalate compounds investigated, 5 were detected in the fin whale samples. DBP, DEP and DEHP were the most abundant compounds, which were detected in 81%, 100% and 58% of fin whale samples, respectively (Table 2, Fig. 2).

The PERMANOVAs fitted including only the individuals of known

Table 2
Median of individual phthalate-compound concentrations expressed in dry weight (dw) and lipid weight (lw), concentration range, and frequency of occurrence (FO) in the muscle of fin whales (n = 31) collected off western Iceland. Non detected compounds were computed as 0.

	dw (μg g^{-1})	lw (μg g^{-1})	Range (μg g^{-1} lw)	FO (%)
DBP	0.303	2.97	<0.0001–23.04	81
DEP	0.303	2.31	0.17–11.42	100
DEHP	0.010	0.07	<0.0001–3.83	58
DMP	0.008	0.07	0.02–0.25	100
DCHP	<0.0001	<0.0007	<0.0007–0.15	3
ΣPAE	799.39	6.01	0.25–37.51	100

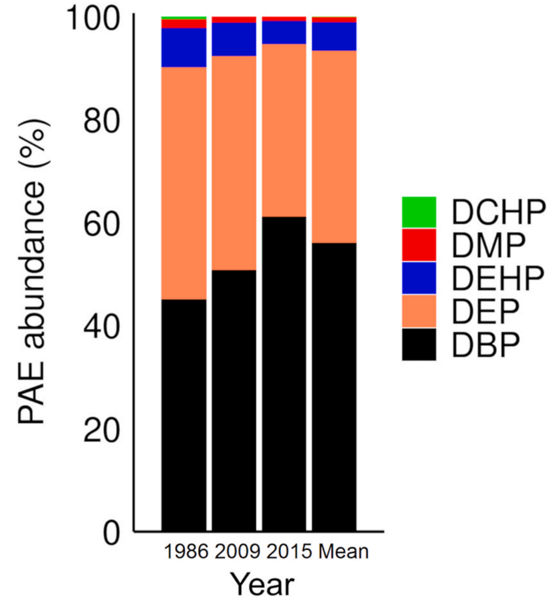

Fig. 2. Percentage contribution of detected PAEs to the total concentration split by year of collection in the muscle of fin whales (n = 31) collected off western Iceland.

Table 3
PERMANOVA results for individual phthalate concentrations (response variables: DBP, DEP, DEHP, DMP, DCHP) ranked by the Akaike information criteria (AIC) including only the fin whales of known age (n = 27) (Type A) or including all the fin whales (n = 31) (Type B) collected off western Iceland. The variables included in the models were: "year" (*i.e.*, year of sample collection; 1986 or 2009–2015), "sex" (*i.e.*, male or female), "length" and "age". The best-fit model for both types is shown in bold. Non detected compounds were computed as 0.

Type	ID	Model	AIC	Explained deviance (%)	Degrees of freedom
A	**MA1**	**Year**	**109.66**	**5.79**	**29**
	MA2	Length	110.36	3.32	29
	MA3	Age	111.05	0.79	29
	MA4	Age + Year	111.10	7.70	28
	MA5	Year + Length	111.15	7.53	28
	MA6	Sex	111.22	0.19	29
	MA7	Year + Sex	111.61	5.93	28
	MA8	Age + Length	112.22	3.79	28
	MA9	Sex + Length	112.26	3.66	28
	MA10	Age + Year + Length	112.67	9.16	27
	MA11	Age + Sex	112.96	1.15	28
	MA12	Year + Sex + Length	113.03	7.93	27
	MA13	Age + Year + Sex	113.06	7.86	27
	MA14	Age + Sex + Length	114.15	4.06	27
	MA15	Age + Year + Sex + Length	114.61	9.38	26
	MA16	Age * Length + Year + Sex	116.21	10.69	25
	MA17	Age * Sex + Year + Length	116.33	10.32	25
B	**MB1**	**Year**	**134.65**	**8.08**	**29**
	MB2	Year + Length	136.38	8.87	28
	MB3	Year + Sex	136.42	8.77	28
	MB4	Length	136.84	1.36	29
	MB5	Sex	137.22	1.36	29
	MB6	Year + Sex + Length	138.07	9.78	27
	MB7	Sex + Length	138.57	2.19	28

age and using all variables plus their interactions did not show any significant effect of age on phthalate concentrations (Table 3; Fig. 3B). As well, the PERMANOVAs fitted including all the individuals showed that none of the explanatory variables (length, sex and year; Fig. 3A, C, D) had a statistically significant effect on phthalate concentrations (Tables 3 and 4), although the variance of phthalate concentrations in the most recent samples (from 2009 to 2015) was higher than that of phthalate concentrations in samples from 1986 (p-value = 0.023, K-squared = 5.158, Barlett test; Fig. 3D).

4. Discussion

This study represents the first investigation on the occurrence and concentration of phthalates in North Atlantic fin whales off western Iceland. We examined the relation between phthalate concentrations and the biological variables of the species and the year of sampling along a period of almost 30 years (from 1986 to 2015).

The only other study on phthalates in North Atlantic fin whales was performed by Routti et al. (2021), who analysed 12 phthalate compounds in the blubber of fin whales from Svalbard, which may transit close to the eastern coast of Iceland during migration (Lydersen et al., 2020). These authors detected only 1 phthalate compound (DEHP) out of the 12 analysed. As these fin whales belong to the same population (Lydersen et al., 2020), differences between studies may be a consequence of comparing different tissues (Sala et al., 2019) or due to analytical methods.

The high incidence of phthalates in the environment (Net et al., 2015) makes it indispensable to control background contamination when analysing biotic phthalate levels. The blank contamination in this study was low (for example <0.012 ng g^{-1} dw for DEHP) compared to other studies (Ikonomou et al., 2012; Routti et al., 2021). Contamination by phthalates can come from many different sources. In the current study, to minimize the potential contamination during the sampling and analysis processes, the use of plastic material was avoided, and all non-volumetric material was rinsed with a suitable solvent just prior to use. Furthermore, the procedure was performed under clean laboratory conditions, using a laminar flow cabinet.

4.1. *Intrapopulation differences*

Phthalates have only recently gained attention as a potentially important group of pollutants affecting marine fauna. This, added to the analytical challenges involved in the determination of these pollutants have also hindered them to be reported (Ikonomou et al., 2012; Net et al., 2015) and, therefore, studies reporting phthalate concentrations in marine mammals are scarce. Moreover, in most of the few studies available, the relationship between phthalate concentrations and the biological variables of the individuals is not assessed, probably as a consequence of reduced sample size and/or of the difficulties in accessing biological information, particularly from free ranging individuals (*e.g.*, Baini et al., 2017; Fossi et al., 2012, 2014, 2016; Hart et al., 2018; Routti et al., 2021).

To the best of our knowledge, the current study provides the first assessment of intrapopulation differences in phthalate concentrations in baleen whales. According to our results, phthalate concentrations in North Atlantic fin whales do not relate to the biological variables of individuals such as age, body length or sex. If this was the case, the different individual exposure to phthalates could be the main cause behind the highly variable concentration ranges reported in the results of the current study. Although the number of samples is not very high, and it would be advisable to have a larger number in order to obtain more conclusive results, the great difficulty of obtaining these samples must be taken into account.

Table 4
Summary of the outputs of the best-fit PERMANOVA, including the variable "year" (MB1), for individual phthalate concentrations (response variables: DBP, DEP, DEHP, DMP, DCHP) in fin whales (n = 31) collected off western Iceland.

Term	Degrees of freedom	Sum of squares	Mean of squares	Pseudo-F	R2	Pr (>F)
Year		5.95	5.95	2.55	0.08	0.08
Residuals		67.66	2.33		0.92	
Total	29	73.61			1	

Three recent studies assessing phthalate concentrations in toothed cetaceans have focused on intrapopulation differences. Dziobak et al. (2021) investigated phthalate metabolites in urinary samples from 51 bottlenose dolphins from Sarasota Bay and concluded that concentrations did not differ between sexes or age classes, a result consistent with the current study. Rian et al. (2020) investigated phthalate metabolites in the liver of 100 harbour porpoises along the coast of Norway and, also consistently with the current study, they did not find any difference between sexes. However, they found a significant negative correlation between the concentration of phthalic acid (a common metabolite of phthalates; Bang et al., 2011) and the body mass and length of the individuals, which was attributed to a more efficient biotransformation process of this metabolite in adult animals (Rian et al., 2020). Page-Karjian et al. (2020) investigated DEP in blubber samples of 46 bottlenose dolphins from Florida and North Carolina and, consistently with the current study, also found no differences between sexes and age classes.

These results appear to be also in line with other studies assessing the relationship between phthalate concentrations and age and body length in other marine vertebrates. Thus, Fourgous et al. (2016) found poor correlations between the concentration of phthalate metabolites in muscle (n = 117) and age and body length of European eels (*Anguilla anguilla*), while Guerranti et al. (2016) found that phthalate concentrations in the muscle of Atlantic bluefin tuna (*Thunnust hynnus*) did not correlate neither to the fish age nor to the fish fork length.

In humans, similarly to what happens in other animals, phthalates do not accumulate in their tissues because they are easily metabolized, and excreted in urine and faeces (Wittassek and Angerer, 2008). However, some authors have reported differences in metabolite concentrations in human populations according to sex and age (*e.g.*, Hartmann et al., 2015; Huang et al., 2015; Silva et al., 2004), which are frequently related to lifestyle and consumer habits (Hartmann et al., 2015; Huang et al., 2015; Parlett et al., 2013), but may also be caused by metabolic differences (Reeves et al., 2019).

However, the absence of bioaccumulation is not necessarily a protection against the deleterious effects of a compound. Although phthalates do not seem to bioaccumulate in fin whale muscles, concern about the potential impact of these compounds on baleen whales should not lessen and monitoring on a medium – large temporal scale is recommended.

4.2. *Temporal differences*

The concentration of phthalates in fin whale muscle did not show any temporal difference during the 29 years span studied (from 1986 to 2015). This result was rather unexpected considering the increase of production and use of phthalates that has occurred during the last decades. Indeed, Net et al. (2015) calculated that phthalate production raised from 1.8 million tons in 1975 to 8 million tons in 2011. This increment parallels the global increase in plastics production (PlasticsEurope, 2016), which raised from 2 million tons in 1950 to 380 million tons in 2015 (Geyer et al., 2017). As a consequence of these trends, it is estimated that, only in 2010, between 4 and 12 million tons of plastic entered the seas (Jambeck et al., 2015). Once in the sea, plastics travel with ocean currents and tend to concentrate in the five subtropical gyres (Cózar et al., 2014) and the Arctic Ocean (Cózar et al., 2017) and, in other regions, close to highly urbanized and industrialized areas and/or in semi-enclosed seas (*e.g.*, Cózar et al., 2015; Lambert

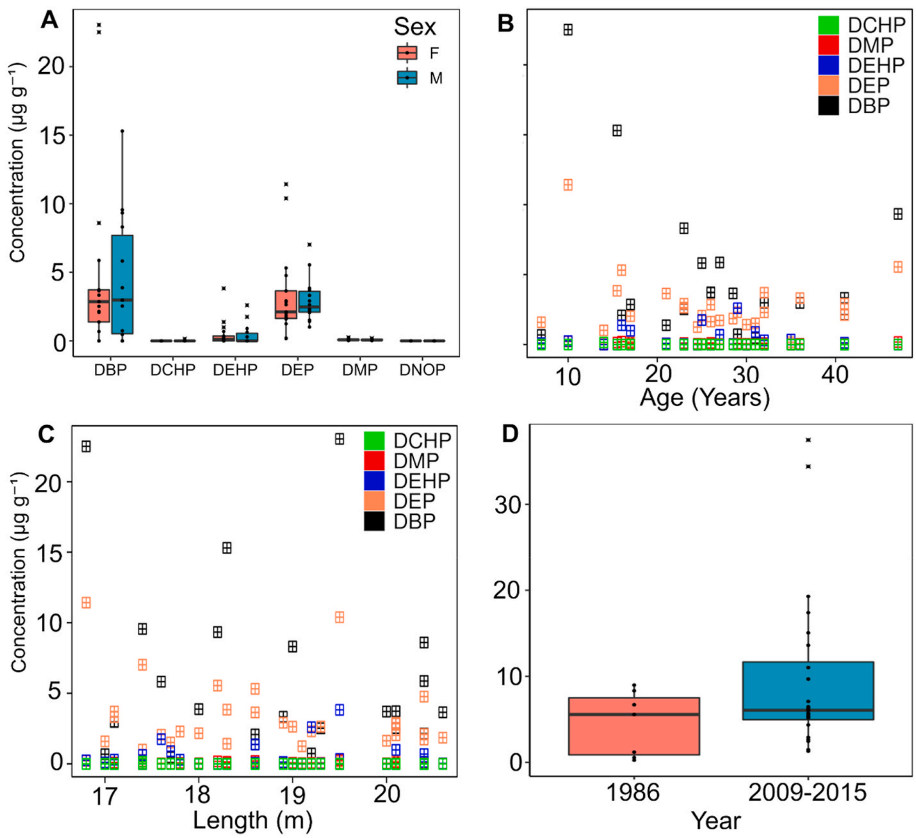

Fig. 3. A: Box-plot illustrating the single phthalate ester (PAE) concentrations in fin whales (n = 31) collected off western Iceland split by sex, B: Scatterplot illustrating the single PAE concentrations in fin whales (n = 27) collected off western Iceland in relation to age of the individual, C: Scatterplot illustrating the single PAE concentrations in fin whales (n = 31) collected off western Iceland in relation to the body length, and D: Box-plot illustrating the total PAE concentrations in fin whales (n = 31) collected off western Iceland split by period of collection (samples from 2009, 2013 and 2015 were grouped). Boxes in A and B subfigures represent the first and third quartiles, lines the median, and vertical bars indicate the most extreme data point which is no more than 1.5 times the interquartile range from the box.

et al., 2020; Ryan, 2014). However, plastics are not the only source of phthalates, as they are present in several materials and products, including building materials, personal-care products, medical devices, detergents and surfactants, packaging, children's toys, printing inks and coatings, pharmaceuticals and food products, textiles, floor tiles, food containers and wrappers, cleaning materials (Net et al., 2015), all of which may contribute to the environmental load of these contaminants.

On the other hand, it can be argued that remote areas, such as the waters off Iceland, are likely to be less polluted by these contaminants, and this might be the cause behind the apparently unnoticeable effects in Icelandic waters of the global trends in plastic and phthalate production. Although the fin whale is an active migrant that undertakes seasonal latitudinal movements spanning large distances Aguilar and García-Vernet, 2018, potentially including areas where phthalate pollution is higher, the results of the current study are consistent with the phthalate concentrations in the atmosphere of the overall North Atlantic Ocean, which in 2017–2018 appear to be similar to that detected during the 1970s (Atlas and Giam, 1981; Bohlin-Nizzetto et al., 2018; Giam et al., 1978). Indeed, Giam et al. (1978) reported DBP and DEHP concentrations ranging 0.4–2.3 and 1.4–4.1 ng m^{-3} respectively in the atmosphere of the North Atlantic Ocean (38°00′N, 69°35′W) during the 1970s, while Bohlin-Nizzetto et al. (2018) reported DBP and DEHP concentrations ranging 0.08–1.01 and 0.29–1.13 ng m^{-3}, respectively, in the Arctic atmosphere, and ranging 0.05–0.20 and 0.15–0.87 ng m^{-3}, respectively, in the North Sea. This would suggest that DBP and DEHP concentrations remained overall constant in the North Atlantic Ocean atmosphere along a period of over 50 years.

Although no significant difference was found between the fin whales sampled in the two distinct periods, the concentration of phthalates in the most recent samples (from 2009 to 2015) included the highest concentrations detected among all the individuals analysed and also showed a higher variance than that of the samples from 1986 (Fig. 3D). It can be hypothesized that the individuals showing the highest concentrations and triggering the increased variance may have visited the North Atlantic subtropical gyre, where marine litter is known to be rapidly accumulating (Cózar et al., 2014). Indeed, the Azores Islands and the south of Portugal, an apparent intermediate stop in autumn destination of fin whales feeding off Iceland in summer (Lydersen et al., 2020; Silva et al., 2013) are located at the margins of the gyre. However, even if the fin whales cross the North Atlantic subtropical gyre during their migratory displacement, the major feeding activity of the species takes place during the summer months in the high-latitude areas such as Iceland (Aguilar and García-Vernet, 2018), where the increase of plastic pollution is less pronounced (Cózar et al., 2015).

The high lability of phthalates may also explain the lack of temporal difference in their concentration in fin whale tissues. These compounds are rapidly degraded in the environment, with photodegradation half-lives (in days) estimated to be 0.38, 0.75, 0.89, 2.39 and 14.41 for DEHP, BBP, DnBP; DEP, and DMP, respectively (Xie et al., 2007). Additionally, the metabolic transformation of phthalates can play an important role on their distribution (Mackintosh et al., 2004), as the metabolic transformation of phthalates in top consumers can cause the consumers to achieve a concentration lower than that in its prey or the environment (Mackintosh et al., 2004). These two factors may combine to determine the environmental availability of phthalates in the ocean and their concentrations in the fin whales' tissues. Whatever the case, the above findings and the hypothesis put forward herein, deserve further research.

From our results we can conclude that: 1) North Atlantic fin whales did not show sex-related differences in phthalate concentrations; and 2) phthalate concentrations did not show temporal differences between the two periods examined (1986 and 2009–2015). Further research is needed to evaluate the pathways of intake and metabolism of phthalates and their potentially toxic effects in this long-lived, filter-feeding and long-range migratory species that, due to these traits, may be highly susceptible to plastic-related pollution.

Credit author statement

Odei Garcia-Garin: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization **Wissam Sahyoun:** Writing – review & editing, Supervision, Methodology **Sopheak Net:** Writing – review & editing, Supervision, Methodology, Funding acquisition **Morgana Vighi:** Writing – review & editing, Supervision **Alex Aguilar:** Writing – review & editing, Supervision, Resources **Baghdad Ouddane:** Writing – review & editing, Funding acquisition **Gísli A. Víkingsson:** Writing – review & editing, Resources **Valerie Chosson:** Writing – review & editing, Resources **Asunción Borrell:** Conceptualization, Writing – review & editing, Supervision, Resources.

Declaration of competing interest

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

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Appendix A. Supplementary data

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DISCUSIÓN

A pesar de los enormes esfuerzos científicos para investigar la problemática de la basura marina y de su impacto en la fauna marina, todavía quedan muchas cuestiones por resolver. Esta tesis utiliza un enfoque interdisciplinar para evaluar la presencia y distribución de las basuras marinas, y sus impactos en vertebrados marinos. Cada capítulo de la tesis aborda una problemática diferente relacionada con la basura marina: macro-residuos flotantes, microplásticos y aditivos plásticos. Los resultados de cada análisis se han contextualizado en las discusiones de cada artículo y en esta sección se discute de manera general la información más relevante de cada capítulo.

El primer capítulo aborda el uso de las nuevas tecnologías para el monitoreo de las basuras marinas flotantes. Se utilizaron avionetas y drones equipados con cámaras de alta resolución. Las imágenes aéreas se analizaron mediante inteligencia artificial y se desarrolló una aplicación web basada en aprendizaje profundo. Los resultados demuestran que la fotografía aérea es una válida alternativa a los métodos tradicionales.

El segundo capítulo aborda el análisis de microplásticos ingeridos, a través del uso de varias especies de vertebrados marinos potencialmente bioindicadoras. El capítulo aborda el análisis de tractos gastrointestinales de bogas del mar Mediterráneo, el análisis del contenido estomacal de rorcuales comunes, y el análisis de los excrementos de lobos marinos antárticos. Los resultados soportan el uso de estas especies como bioindicadoras de contaminación marina por microplásticos.

El tercer capítulo aborda el análisis de aditivos plásticos en tejido muscular de vertebrados marinos. Esto se hizo mediante el análisis de ésteres organofosforados y ftalatos en tejido de bogas y rorcuales comunes. Se encontraron ésteres organofosforados en el tejido de ambas especies, además de ftalatos en los rorcuales comunes.

Abundancia, distribución y composición de la basura marina

Monitorear la abundancia, y los patrones de distribución y composición de la basura en el océano mediante metodologías estandardizadas es vital para revertir esta amenaza global (GESAMP, 2015; UNEP, 2016). En el marco general de las normativas medioambientales (p. ej., la Directiva Marco sobre la Estrategia Marina (MSFD, por sus siglas en inglés) 2008/56/EC) es crucial definir los niveles basales de basura marina. En la presente tesis se han investigado diversos aspectos de la basura marina: macro-residuos flotantes, microplásticos y aditivos plásticos.

Los macro-residuos flotantes se han investigado mediante drones y avionetas equipados con cámaras, y mediante observaciones visuales desde barcos y avionetas (Tabla 1). Mediante drones, se detectaron densidades de 0 a 200 macro-residuos flotantes km^{-2}. Además, se identificaron diferencias en la concentración de macro-residuos flotantes entre áreas de la costa catalana: las áreas marinas adyacentes a Barcelona y al Delta de l'Ebre presentaron densidades superiores que el área marina del Cap de Creus. Mediante fotografía aérea desde avioneta, se detectaron densidades de 0 a 10 macro-residuos flotantes km^{-2} en el área marina que va desde el Delta de l'Ebre hasta Alicante. Mediante observaciones visuales desde barco, se detectaron densidades de macro-residuos flotantes de 0 a 70 objetos km^{-2} en las aguas marinas adyacentes a la ciudad de Barcelona. Mediante observaciones visuales desde avioneta, se detectaron densidades de macro-residuos flotantes de 0 a 5 objetos km^{-2} en el área marina que va desde el Delta de l'Ebre hasta Alicante. Además, se detectó un gradiente positivo de densidad de macro-residuos flotantes respecto a la cercanía a la costa. Los principales objetos detectados fueron plásticos no identificados, bolsas de plástico, agregaciones de plástico, redes de pesca, boyas, cajas, láminas de plástico y botellas. Estos resultados identifican la costa catalana como altamente contaminada por macro-residuos flotantes en comparación con los resultados reportados en la misma área por estudios que utilizaron observaciones visuales desde barcos y avionetas (Arcangeli et al., 2018; Lambert et al., 2020; Suaria & Aliani, 2014). Además, los resultados de composición fueron coherentes con otros estudios que reportan proporciones de más del 80% de plástico en los macro-residuos flotantes (Arcangeli et al., 2018; Campanale et al., 2019; Galgani et al., 2015; UNEP/ MAP, 2015).

La abundancia, distribución y composición de microplásticos en aguas marinas se ha investigado mediante especies potencialmente bioindicadoras de contaminación por basuras marinas (boga, rorcual común y lobo marino antártico). Se encontraron concentraciones de microplásticos de 0,16 - 0,83; 0,057 y 0 microplásticos g^{-1} en bogas de la costa catalana (Tabla 1), rorcuales comunes que se alimentan en las aguas de Islandia y en excrementos de lobos marinos antárticos que se alimentan en las aguas del Estrecho de Bransfield (Antártida), respectivamente. Estos resultados, partiendo del hecho que estas especies han sido propuestas como potenciales bioindicadoras de contaminación marina (Fossi et al., 2018; Pérez-Venegas et al., 2020; Tsangaris et al., 2020), son un indicador de la abundancia y composición de los microplásticos presentes en estas áreas marinas. Los resultados han permitido identificar la costa catalana, y especialmente las aguas adyacentes a la ciudad de Barcelona, como una zona de alta concentración de microplásticos. Estos resultados eran esperables ya que el mar Mediterráneo es una de las zonas más contaminadas del mundo por la basura marina (Cózar et al., 2015), y, concretamente, la ciudad de Barcelona es una de las ciudades que aporta más basura al mar Mediterráneo, con una contribución anual de 1800 toneladas (Liubartseva et al., 2018). Los principales polímeros detectados (excluyendo las fibras modificadas de celulosa) analizando las partículas encontradas en los tres estudios fueron polipropileno, polietileno y poliestireno. Este resultado era esperable, ya que estos tres compuestos son los polímeros más abundantes en los plásticos presentes en la columna de agua de los mares y océanos de todo el mundo (Suaria et al., 2016; Cózar et al., 2017). La mayoría de los microplásticos detectados en la costa catalana fueron fragmentos (60%) y, por el contrario, los detectados en las aguas de Islandia fueron mayormente fibras (69%), resultado que probablemente está relacionado con las fuentes locales de microplásticos.

Tabla 1: Rango de concentraciones de microplásticos (MP) y de densidades de macro-residuos en la costa Mediterránea de España en función de la metodología usada.

Metodología	Rango de concentraciones de microplásticos (MP g^{-1})	Rango de densidad de macro-residuos (objetos km^{-2})
Dron - fotografía aérea	-	0 - 200
Avioneta - fotografía aérea	-	0 - 10
Avioneta - observadores	-	0 - 5
Barco - observadores	-	0 - 70
Bogas – contenido estomacal	0,16 - 0,83	-

Los aditivos plásticos se han investigado en tejido muscular de vertebrados marinos (bogas y rorcuales comunes). En bogas de la costa catalana, se detectaron concentraciones de ésteres organofosforados de 0 a 2566 ng g^{-1} en peso lipídico. Además, las bogas pescadas en las aguas adyacentes a Barcelona presentaron concentraciones superiores de aditivos plásticos respecto a las bogas que procedían de la reserva marina del Cap de Creus. Este resultado concuerda con la mayor densidad de microplásticos en las bogas procedentes de Barcelona, aunque no se observó una correlación entre las dos variables. En rorcuales comunes de Islandia, se detectaron concentraciones de ésteres organofosforados y ftalatos de 31,9 a 10232 y de 245 a 37514 ng g^{-1} en peso lipídico, respectivamente. Estos resultados parecen indicar que las aguas de Islandia están más contaminadas por aditivos plásticos que las aguas de la costa catalana, aunque se tiene que tener en cuenta que se ha estimado que los rorcuales comunes de Islandia ingieren de 38646 a 77292 microplásticos por día, cosa que implica que esta especie está mucho más expuesta a la absorción de aditivos plásticos en sus tejidos respecto a la boga.

Los resultados obtenidos en la presente tesis aportan datos de abundancia, distribución y composición de macro-residuos flotantes en el Mediterráneo noroccidental, microplásticos y aditivos plásticos en especies indicadoras. Conocer los niveles basales de basura marina es crucial y relevante en caso que hayan pocos datos previos (como en el caso de la Antártida) y para detectar posibles tendencias temporales. Los resultados describen los niveles de basura marina actuales y, por lo tanto, son relevantes porque en un futuro podrán servir para determinar si las medidas de mitigación (p. ej., la prohibición de ciertos plásticos de un solo uso o de ciertos aditivos plásticos) han sido eficaces en disminuir los niveles de basura marina o, por lo contrario, sigue la tendencia ascendente de contaminación plástica. Por otro lado, es muy importante tener en cuenta la metodología utilizada para comparar niveles de basura marina obtenidos en diferentes regiones o épocas, ya que podrían estar influyendo en los resultados (Zantis et al., 2021b).

Potenciales impactos de la basura marina sobre la fauna marina

En la presente tesis se ha investigado el potencial impacto de la basura marina sobre los vertebrados marinos mediante el análisis de su ocurrencia y concentración en 3 especies: boga, rorcual común y lobo marino antártico.

Las investigaciones realizadas en tractos digestivos y muestras de tejido muscular de bogas de la costa catalana determinaron que la boga es una especie que ingiere microplásticos (ocurrencia del 46%) y que presenta niveles considerables de aditivos plásticos (ésteres organofosforados) en sus tejidos. Otros estudios de microplásticos en bogas también reportan valores de ocurrencia similares (Nadal et al., 2016; Rios-fuster et al., 2019; Tsangaris et al., 2020), aunque Neves et al. (2015) encontró microplásticos solamente en el 9% de las bogas muestreadas a lo largo de la costa portuguesa. Aunque las comparaciones entre estudios pueden estar sesgadas por el tipo de metodología analítica, estos resultados pueden sugerir que la ingesta de microplásticos puede reflejar diferencias ambientales en los niveles de estos contaminantes (Tsangaris et al., 2020). Por este motivo, la boga se ha propuesto como especie bioindicadora de los niveles de microplásticos en el medio marino (Tsangaris et al., 2020). Por otro lado, los niveles de aditivos plásticos presentes en sus tejidos fueron 5 órdenes de magnitud inferiores a la dosis media letal en animales de laboratorio (Du et al., 2019; van der Veen and de Boer, 2012) y, por lo tanto, el impacto de los ésteres organofosforados en las bogas parece ser limitado. Las concentraciones de ésteres organofosforados detectados en otras especies de peces (sardina (*Sardina pilchardus*), boquerón (*Engraulis encrasicolus*) y merluza (*Merluccius merluccius*)) en el mar Mediterráneo también son relativamente bajas y tampoco parecen suponer un riesgo para estos animales o para la salud humana mediante su consumo (Sala et al., 2022).

Las investigaciones realizadas en muestras de contenido estomacal y de tejido muscular de rorcual común del Atlántico norte determinaron que esta especie ingiere grandes cantidades de partículas sintéticas diariamente, y que presenta en sus tejidos aditivos plásticos (ésteres organofosforados y ftalatos) altamente tóxicos. Otros estudios sobre la misma población de rorcuales comunes indican que también está afectada por otros contaminantes orgánicos persistentes como los PCBs y DDTs (Borrell, 1993), y otros aditivos plásticos como los PBDEs (Rotander et al., 2012). Aunque aún no se ha descrito ningún efecto tóxico de estos contaminantes sobre los rorcuales comunes, estudios en mamíferos marinos, como delfines y focas, que pertenecen a niveles tróficos superiores, muestran que la acumulación de contaminantes orgánicos persistentes en sus tejidos puede provocar efectos negativos en la reproducción y efectos inmunosupresores (Aguilar y Borrell, 1994; Jepson et al., 2016; Reijnders, 1986). Se debe tener en cuenta que en la presente tesis se han determinado los niveles de microplásticos y aditivos plásticos en rorcuales comunes del Atlántico norte, pero el riesgo y los efectos adversos que pueden producir estos contaminantes en esta especie son aún desconocidos.

La investigación realizada en excrementos de lobo marino antártico recolectados en la isla Decepción (oeste de la Antártida) determinó que la población de esta especie, que se alimenta en el Estrecho de Bransfield durante el verano austral, está muy poco afectada por los microplásticos. Por lo contrario, se encontraron microplásticos en excrementos de lobo marino antártico de la Isla Macquarie, con unos niveles de 1 a 4 microplásticos por excremento (Eriksson y Burton, 2003). Pérez-Venegas et al. (2020) propusieron que los excrementos de los pinnípedos podían ser un buen indicador de los niveles de microplásticos en las aguas donde se alimentan, por lo que la ausencia de microplásticos en los excrementos de lobo marino antártico indicarían que los niveles de microplásticos en las aguas de la Isla Decepción y sus alrededores son extremadamente bajos.

Metodologías para monitorear las basuras y sus impactos en la fauna marina: ventajas y desventajas

En la presente tesis se han usado diversas metodologías para investigar diferentes aspectos de las basuras marinas: vuelos con avioneta equipada con cámaras de alta resolución y observadores, transectos visuales desde barco y transectos mediante dron, técnicas de aprendizaje profundo mediante redes neuronales convolucionales, análisis de microplásticos tanto en el tracto digestivo de peces, como en el contenido estomacal de ballenas y en excrementos de lobo marino, y análisis químicos de aditivos plásticos en tejido muscular de peces y ballenas.

Los resultados obtenidos en el primer capítulo han demostrado que las técnicas de teledetección (drones y avionetas equipados con cámaras) pueden reemplazar las metodologías tradicionales (observaciones visuales desde avioneta, desde barcos, etc.) de monitoreo de macro-residuos flotantes y fauna marina. Además, las técnicas de teledetección enlazadas con algoritmos de aprendizaje profundo pueden mejorar las prestaciones de este tipo de monitoreo. Estas técnicas de teledetección presentan claros beneficios respecto a las técnicas de monitoreo tradicionales: (1) Las imágenes se pueden reanalizar varias veces por múltiples operadores o mediante algoritmos automatizados, reduciendo el error humano; (2) el área de monitoreo y el tamaño de los macro-residuos o de la fauna marina se pueden determinar con precisión; y (3) las imágenes se pueden usar a nivel multidisciplinar para investigar diferentes cuestiones. A pesar de esto, también presentan algunas desventajas: (1) los transectos visuales pueden cubrir áreas más extensas y son menos costosos; (2) existen limitaciones de vuelo debidas a legislaciones locales; (3) la dificultad de detectar y cuantificar los macro-residuos y la fauna marina en las imágenes debido a la variedad de materiales, objetos, colores, y a las condiciones ambientales (Andriolo et al., 2022; Arcangeli et al., 2020; Colefax et al., 2017; Deidun et al., 2018; Fiori et al., 2017; Gonçalves et al., 2020b). No obstante, las ventajas de las nuevas tecnologías superan claramente las desventajas y obstáculos que presentan (Andriolo et al., 2022) y, por esta razón, es prioritario seguir desarrollando y mejorando las técnicas de teledetección para poder monitorear los macro-residuos marinos a escala global y en intervalos repetidos en el tiempo.

Además, los resultados del segundo capítulo han avalado el uso de especies bioindicadoras para determinar los niveles de microplásticos en el medio marino. El uso de la boga como especie bioindicadora de los niveles de microplásticos en el medio bentopelágico marino ha sido avalada por varios estudios científicos (Bray et al., 2019;

Tsangaris et al., 2020), y también por los resultados del proyecto europeo MEDSEALITTER (MEDSEALITTER consortium, 2019), cuyo objetivo era desarrollar metodologías para el monitoreo de la basura marina. El uso de rorcuales comunes como bioindicadores de microplásticos ya había sido propuesto por otros estudios (Baini et al., 2017; Fossi et al., 2014, 2018). Los rorcuales son animales de gran tamaño que se alimentan principalmente de krill y, por lo tanto, necesitan filtrar grandes cantidades de agua para satisfacer sus necesidades energéticas (Aguilar y García-Vernet, 2018). De esta manera, es muy probable que durante las filtraciones que realizan para alimentarse ingieran una muestra representativa de los microplásticos presentes en el medio pelágico marino (Fossi et al., 2018). Finalmente, el uso de excrementos de pinnípedos como bioindicadores de los microplásticos presentes en el medio marino también ha sido respaldado por estudios recientes (Pérez-Venegas et al., 2020). La ventaja principal del uso de excrementos es que su recogida no perturba al animal (técnica no invasiva) (Nelms et al., 2019; Pérez-Venegas et al., 2020). Es esencial que el análisis de microplásticos vaya acoplado a la espectroscopía de infrarrojos transformada de Fourier (FTIR), ya que esta permite determinar el tipo de polímero de los plásticos detectados y, de esta manera, inferir la fuente de origen (Tsangaris et al., 2020).

Recientemente, el Plan de Acción para el Mediterráneo del Programa de las Naciones Unidas para el Medio Ambiente (UNEP/ MAP) reportó que para monitorear los impactos de la basura en la fauna marina es necesario tener a disposición especies bioindicadoras que permitan determinar tanto la ocurrencia de la basura marina como los efectos de su ingestión (Galgani, 2017). Además, también se sugirió que el monitoreo se debería realizar mediante diversas especies bioindicadoras, cada una de las cuales fuera específica para un tipo de basura (plástico, madera, metal, etc.), y de un compartimento marino (superficie, columna de agua, bentos), teniendo en cuenta sus hábitos alimenticios (Galgani, 2017). Actualmente, la convención de Oslo y Paris para la protección del medio marino del noreste Atlántico (OSPAR) utiliza el fulmar boreal (*Fulmarus glacialis*) como especie bioindicadora para monitorear la basura marina. Además, la UNEP/ MAP ha recomendado el uso de la tortuga boba (*Caretta caretta*) como especie bioindicadora para monitorear los macro-residuos marinos (UNEP/ IMAP, 2017).

Finalmente, en el tercer capítulo de la tesis se han usado técnicas cromatográficas para detectar y cuantificar ésteres organofosforados (LC-MS/MS) en tejido muscular de rorcuales comunes y de bogas, y ftalatos (GC-MS/MS) en tejido muscular de rorcuales comunes. Estas técnicas son de gran precisión y fiabilidad y se han usado tradicionalmente para detectar y cuantificar otros contaminantes orgánicos persistentes como los PBDEs (Aznar-Alemany et al., 2021; Capanni et al., 2020) y los PCBs (Jepson et al., 2016; Panti et al., 2022), en mamíferos marinos. El inconveniente principal de trabajar con aditivos plásticos es la posible contaminación de las muestras (Ikonomou et al., 2012; Routti et al., 2021). Por esta razón, las condiciones de trabajo deben ser muy limpias, se tiene que trabajar bajo una campana de flujo laminar, utilizar blancos en todas las etapas del método y evitar los materiales de plástico (Sala et al., 2022). El nivel de uso de estas técnicas es avanzado y la mayoría de estudios usan una metodología estandardizada (p. ej., Sala et al., 2022; Routti et al., 2021). El % de recuperación de los compuestos analizados, el límite de detección y el límite de cuantificación son reportados habitualmente en estudios de aditivos plásticos en biota (Routti et al., 2021).

Perspectivas futuras

La contaminación por basura marina es uno de los principales problemas de la sociedad actual. La basura marina se encuentra en todos los compartimentos de los mares y océanos de la Tierra, y muchas veces se acumula en las zonas más prístinas y remotas debido a su transporte por las corrientes marinas y a que no se realizan acciones de limpieza en las islas o áreas deshabitadas. Así pues, además de los compartimentos estudiados en la presente tesis, también es de vital importancia su estudio y monitoreo en playas y bentos marino, donde la basura marina se acumula en grandes cantidades.

En la Unión Europea, hay una normativa relativamente avanzada para lidiar con la problemática de la basura. La MSFD sugiere y requiere la monitorización de la basura marina para la evaluación continua del estado del medio marino. Además, la Directiva de Plásticos de un Solo Uso (EU) 2019/904, la Directiva de Bolsas de Plástico (EU) 2015/720 y la Directiva 2018/851 tienen como objetivos reducir la producción y el consumo de plásticos y consecuentemente la producción de residuos, y también la implementación de programas de monitoreo para evaluar las medidas propuestas. Para cumplir con las directivas y, además, aportar datos que ayuden a evaluar su eficacia, son indispensables más estudios, como los reportados en la presente tesis, que evalúen el estado de contaminación por basura en el medio marino. Es crucial que estos estudios sean estandardizados a través de una calibración previa de los resultados obtenidos mediante diferentes metodologías (UNEP, 2021), y que las metodologías usadas entre los distintos proyectos, grupos de investigación o países sean estándares para que los resultados sean comparables.

Además, también son necesarios estudios aplicados para desarrollar y mejorar las técnicas de detección, cuantificación y limpieza del medio marino. Por ejemplo, son necesarios avances para la integración de los algoritmos de inteligencia artificial directamente en sensores remotos como drones, cámaras de alta resolución o satélites para automatizar aún más el proceso de detección y cuantificación de los macro-residuos. De hecho, recientemente se han empezado a usar las imágenes satelitales para detectar y cuantificar la basura marina (Topouzelis et al., 2019). Además, también sería interesante que la tecnología aérea pudiera enviar información a drones acuáticos que se desplazaran automáticamente a recoger la basura marina previamente detectada. Por otra parte, son necesarias más investigaciones sobre los riegos potenciales de los aditivos plásticos y microplásticos en la fauna marina (Burns y Boxall, 2018), ya que solamente evaluando su presencia en los tejidos de la fauna marina se desconocen sus potenciales efectos.

Por estas razones, la problemática de la basura marina requiere de investigaciones integradas e interdisciplinares mediante la cooperación entre especialistas de la temática, incluyendo todos los sectores implicados (académicos, especialistas de la industria, etc.) (UNEP, 2021). Es evidente que hay un largo camino por recorrer, los plásticos están presentes en todos los ámbitos de nuestra vida cotidiana y las basuras siguen acumulándose en los mares y océanos en cantidades desorbitadas. Solo promoviendo las colaboraciones entre grupos de expertos, el trabajo con los *stakeholders*, e inviirtiendo en ciencia y tecnología, se podrá empezar a revertir la gran problemática de la basura marina.

CONCLUSIONES

- The drone-based method proved to be as valid as the observer-based method for monitoring floating marine macro-litter.
- The photographic method proved to be as valid as the observer-based method for monitoring floating marine macro-litter and marine megafauna.
- Manned aerial vehicles are more suitable for floating macro-litter monitoring at large spatial scales, while drones are more suitable for its monitoring at relatively small-scales and/or when more accurate data are needed.
- Our results indicated that the waters off the city of Barcelona are heavily polluted by marine litter, and so is the "Delta de l'Ebre" area, where the density of floating marine litter observed was even higher, probably due to the inputs from the Ebro river.
- The deep learning model developed, based on a convolutional neural network connected to a web-oriented application on the Shiny package, showed great efficiency in the automatic detection and quantification of floating marine litter in aerial images, which promises great advances compared to traditional methods.
- The use of the bogue as a bioindicator of contamination by microplastics was successful in the Mediterranean Sea, and results of the analyses of its stomach content identified the marine area off Barcelona as an area of concentration of microplastics.
- The large number of synthetic particles detected in the stomachs of North Atlantic fin whales showed that this species may be vulnerable to this type of pollution.
- The absence of microplastics in the scats of male Antarctic fur seals on Deception Island suggested that the Bransfield Strait food web is virtually pristine.
- Despite the lack of correlation between the concentration of organophosphate esters and the number of microplastics in the gastrointestinal tract of bogues, the high concentration of these pollutants in the waters off Barcelona indicated that it is potentially a microplastic concentration zone.
- The results obtained from the analysis of the muscle of fin whales and their prey (krill) indicate that organophosphate esters do not appear to biomagnify or bioaccumulate.
- Concentrations of phthalates in the muscle of North Atlantic fin whales have not increased in the last 30 years, and therefore do not seem to pose an imminent danger to the North Atlantic fauna.
- The results of this thesis can serve to provide reference values and to improve the monitoring of marine litter.
- For a complete assessment of this issue, marine litter on beaches and seabed should also be assessed, and other technologies or methods, such as satellites, to combat marine pollution in the global ocean should be taken into consideration.

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