Universidad de Oviedo

Programa Oficial de Doctorado en Ingeniería Química, Ambiental y Bioalimentaria

Cuantifación e impacto de microplásticos en especies marinas

Quantification and impact of microplastics in marine species

Departamento de Biología Funcional, Área de Genética

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TESIS DOCTORAL

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RESUMEN (en español)

Los microplásticos son un contaminante ubicuo en todos los ecosistemas donde se han estudiado, y por sus características físicas y las sustancias químicas que pueden llevar adheridas son un peligro para las especies expuestas a ellos. La presente Tesis tiene como objetivo la evaluación del impacto de los microplásticos en diferentes especies marinas de interés comercial. Para ello, se han estudiado organismos de diferentes niveles tróficos, comenzando por las algas rojas que son la base de la industria gelificante. Tras analizar las distintas etapas de la cadena de producción de agentes gelificantes como el agar y la carragenina, se han identificado puntos clave en los que puede ocurrir contaminación por microplásticos: durante su recogida y manejo, a lo largo del procesamiento industrial y en el proceso de gestión postindustrial de los residuos generados.

La siguiente especie estudiada, está en peligro crítico de extinción: la anguila europea (*Anguilla anguilla*). Se cuantificaron microplásticos en juveniles (etapa de angula de cristal) en el momento de llegada a tres ríos de Asturias (sur del Golfo de Vizcaya): Nalón, Bedón y Cabra. Se ha evidenciado que son portadoras de microplásticos insertados dentro del tejido muscular, probablemente incorporado durante el desarrollo temprano en el Mar de los Sargazos, demostrando así la movilización de microplásticos desde el mar hacia los ríos opuesta a la bien conocida direccionalidad río-mar de estos contaminantes. Además, se ha comprobado que acumulan partículas procedentes del río, que se adhieren a su superficie en una concentración de varios órdenes de magnitud superior al medio circundante. Entre ellas se han identificado polímeros de gran toxicidad para la vida acuática como el poliéster o el poliacrilonitrilo, entre otros. Esta exposición a microplásticos podría contribuir al declive de la vulnerable población de anguila europea.

Por otra parte, en esta Tesis se ha evidenciado por primera vez el impacto producido por los microplásticos en la especie de merluza demersal *Merluccius polli* (merluza negra o de Benguela). Esta especie se distribuye en el Atlántico centro-oriental (oeste de África), donde además de contribuir a las pesquerías locales es explotada por la flota pesquera europea dentro de los Acuerdos de Partenariado para Pesquerías Sostenibles de la Unión Europea con países africanos (Sustainable Fisheries Partnership

Agreements). Se encontraron microplásticos en branquias, músculo e hígado de todas las merluzas analizadas, siendo tóxicos algunos polímeros como el poliéster o el poliacrilonitrilo. Se ha constatado un efecto negativo de los microplásticos sobre el estado fisiológico de las merluzas, reflejado en una correlación negativa entre el factor de condición y la concentración de microplásticos en el hígado. Se encontró también una correlación positiva entre esta concentración de microplásticos y el grado de degradación del ADN genómico, interpretada en este caso como un daño físico a las células durante el proceso de descompresión al que están sometidas las merluzas al subirlas a la superficie durante la pesca.

Finalmente, se proponen medidas para eliminar o, al menos, reducir el aporte de microplásticos a los compartimentos acuáticos de la biosfera que terminarán acumulándose en mares y océanos. Entre ellas, desde el punto de vista técnico, es imprescindible la mejora de los sistemas de retención de microplásticos en las estaciones depuradoras de aguas residuales. En la vertiente social, la educación medioambiental generaría concienciación colectiva sobre el problema y ayudaría a buscar soluciones de manera comunitaria y efectiva, comenzando por el reciclaje y el cambio en los hábitos de consumo. Por último, se presenta la necesidad del desarrollo y aplicación de políticas sostenibles y eficaces a nivel internacional que controlen el vertido de plásticos a los ríos y mares, comenzando por los plásticos de un solo uso y la adición de microesferas en productos de higiene y limpieza.

RESUMEN (en Inglés)

Microplastics are an ubiquitous contaminant in all the ecosystems where they have been studied. Due to their physical characteristics and the chemical substances that can be attached to them, they are a danger to the species exposed to them. The objective of this thesis is to evaluate the impact of microplastics on different marine species of commercial interest. For this, organisms of different trophic levels have been studied, beginning with the red algae that are the basis of the gelling industry. After analyzing the different stages of the production chain of gelling agents such as agar and carrageenan, key points have been identified where contamination by microplastics can occur: during collection and handling, throughout industrial processing and in the post-industrial management process of generated waste.

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The next species studied is critically endangered: the European eel (*Anguilla anguilla*). Microplastics were quantified in juveniles (glass eel stage) at the time of arrival in three rivers in Asturias (southern Bay of Biscay): Nalón, Bedón and Cabra. It has been shown that they are carriers of microplastics inserted into the muscle tissue, probably incorporated during early development in the Sargasso Sea, thus demonstrating the mobilization of microplastics from the sea to the rivers, contrary to the well-known river-sea directionality of these contaminants. In addition, glass eels accumulate particles from the river, which adhere to its surface in a concentration several orders of magnitude higher than the surrounding environment. Among them, polymers of great toxicity for aquatic life such as polyester or polyacrylonitrile, among others, have been identified. This exposure to microplastics could contribute to the decline of the vulnerable European eel population.

On the other hand, in this thesis, the impact produced by microplastics on the demersal species *Merluccius polli* (black or Benguela hake) has been evidenced for the first time. This species is distributed in the Central-Eastern Atlantic (West Africa), where, in addition to contributing to local fisheries, it is exploited by the European fishing fleet within the Sustainable Fisheries Partnership Agreements of the European Union with African countries. Microplastics were found in the gills, muscle and liver of all the analyzed hakes, some of which were toxic polymers such as polyester or polyacrylonitrile. A negative effect of microplastics on the physiological state of hake has been found, reflected in a negative correlation between the condition factor and the concentration of microplastics in the liver. A positive correlation was also found between this concentration of microplastics and the degree of degradation of the genomic DNA, interpreted in this case by physical damage to the cells during the decompression process to which the hakes are subjected when they are hauled to the surface during fishing.

Finally, measures are proposed to eliminate or, at least, reduce the arrival of microplastics to the aquatic compartments of the biosphere that will end up accumulating in seas and oceans. Among them, from a technical point of view, it is essential to improve microplastic retention systems in wastewater treatment plants. On the social side, environmental education would generate collective awareness of the problem and would help to find solutions in a communal and effective way, starting with recycling and changing consumption habits. Finally, it is necessary to develop and apply sustainable and effective policies at an international level that control the dumping of plastics into rivers and seas, beginning with single-use plastics and the addition of microspheres in hygiene and cleaning products.

SR. PRESIDENTE DE LA COMISIÓN ACADÉMICA DEL PROGRAMA DE DOCTORADO EN ____________________

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Índice

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Resumen

Los microplásticos son un contaminante ubicuo en todos los ecosistemas donde se han estudiado, y por sus características físicas y las sustancias químicas que pueden llevar adheridas son un peligro para las especies expuestas a ellos. La presente Tesis tiene como objetivo la evaluación del impacto de los microplásticos en diferentes especies marinas de interés comercial. Para ello, se han estudiado organismos de diferentes niveles tróficos, comenzando por las algas rojas que son la base de la industria gelificante. Tras analizar las distintas etapas de la cadena de producción de agentes gelificantes como el agar y la carragenina, se han identificado puntos clave en los que puede ocurrir contaminación por microplásticos: durante su recogida y manejo, a lo largo del procesamiento industrial y en el proceso de gestión postindustrial de los residuos generados.

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Por otra parte, en esta Tesis se ha evidenciado por primera vez el impacto producido por los microplásticos en la especie de merluza demersal *Merluccius polli* (merluza negra o de Benguela). Esta especie se distribuye en el Atlántico centro-oriental (oeste de África), donde además de contribuir a las pesquerías locales es explotada por la flota pesquera europea dentro de los Acuerdos de Partenariado para Pesquerías Sostenibles de la Unión Europea con países africanos (Sustainable Fisheries Partnership Agreements). Se encontraron microplásticos en branquias, músculo e hígado de todas las merluzas analizadas, siendo tóxicos algunos polímeros como el poliéster o el poliacrilonitrilo. Se ha constatado un efecto negativo de los microplásticos sobre el estado fisiológico de las merluzas, reflejado en una correlación negativa entre el factor de condición y la concentración de microplásticos en el hígado. Se encontró también una correlación positiva entre esta concentración de microplásticos y el grado de degradación del ADN genómico, interpretada en este caso como un daño físico a las células durante el proceso de descompresión al que están sometidas las merluzas al subirlas a la superficie durante la pesca.

Finalmente, se proponen medidas para eliminar o, al menos, reducir el aporte de microplásticos a los compartimentos acuáticos de la biosfera que terminarán acumulándose en mares y océanos. Entre ellas, desde el punto de vista técnico, es imprescindible la mejora de los sistemas de retención de microplásticos en las estaciones depuradoras de aguas residuales. En la vertiente social, la educación medioambiental generaría concienciación colectiva sobre el problema y ayudaría a buscar soluciones de manera comunitaria y efectiva, comenzando por el reciclaje y el cambio en los hábitos de consumo. Por último, se presenta la necesidad del desarrollo y aplicación de políticas sostenibles y eficaces a nivel internacional que controlen el vertido de plásticos a los ríos y mares, comenzando por los plásticos de un solo uso y la adición de microesferas en productos de higiene y limpieza.

Summary

Microplastics are an ubiquitous contaminant in all the ecosystems where they have been studied. Due to their physical characteristics and the chemical substances that can be attached to them, they are a danger to the species exposed to them. The objective of this thesis is to evaluate the impact of microplastics on different marine species of commercial interest. For this, organisms of different trophic levels have been studied, beginning with the red algae that are the basis of the gelling industry. After analyzing the different stages of the production chain of gelling agents such as agar and carrageenan, key points have been identified where contamination by microplastics can occur: during collection and handling, throughout industrial processing and in the post-industrial management process of generated waste.

The next species studied is critically endangered: the European eel (*Anguilla anguilla*). Microplastics were quantified in juveniles (glass eel stage) at the time of arrival in three rivers in Asturias (southern Bay of Biscay): Nalón, Bedón and Cabra. It has been shown that they are carriers of microplastics inserted into the muscle tissue, probably incorporated during early development in the Sargasso Sea, thus demonstrating the mobilization of microplastics from the sea to the rivers, contrary to the well-known river-sea directionality of these contaminants. In addition, glass eels accumulate particles from the river, which adhere to its surface in a concentration several orders of magnitude higher than the surrounding environment. Among them, polymers of great toxicity for aquatic life such as polyester or polyacrylonitrile, among others, have been identified. This exposure to microplastics could contribute to the decline of the vulnerable European eel population.

On the other hand, in this thesis, the impact produced by microplastics on the demersal species *Merluccius polli* (black or Benguela hake) has been evidenced for the first time. This species is distributed in the Central-Eastern Atlantic (West Africa), where, in addition to contributing to local fisheries, it is exploited by the European fishing fleet within the Sustainable Fisheries Partnership Agreements of the European Union with African countries. Microplastics were found in the gills, muscle and liver of all the analyzed hakes, some of which were toxic polymers such as polyester or polyacrylonitrile. A negative effect of microplastics on the physiological state of hake has been found, reflected in a negative correlation between the condition factor and the concentration of microplastics in the liver. A positive correlation was also found between this concentration of microplastics and the degree of degradation of the genomic DNA, interpreted in this case by physical damage to the cells during the decompression process to which the hakes are subjected when they are hauled to the surface during fishing.

Finally, measures are proposed to eliminate or, at least, reduce the arrival of microplastics to the aquatic compartments of the biosphere that will end up accumulating in seas and oceans. Among them, from a technical point of view, it is essential to improve microplastic retention systems in wastewater treatment plants. On the social side, environmental education would generate collective awareness of the problem and would help to find solutions in a communal and effective way, starting with recycling and changing consumption habits. Finally, it is necessary to develop and apply sustainable and effective policies at an international level that control the dumping of plastics into rivers and seas, beginning with single-use plastics and the addition of microspheres in hygiene and cleaning products.

Introducción

1. Descubriendo el plástico

1.1. Breve historia del plástico

De entre todos los potenciales contaminantes que pueden ser estudiados en el medio marino, la presente Tesis se focaliza en un tipo de partículas emergentes cuya presencia en los océanos es alarmantemente creciente: los microplásticos. Independientemente del prefijo *micro-* utilizado en este concepto, y para contextualizar históricamente la problemática que se presenta en este manuscrito, se va a realizar un pequeño repaso en torno a la historia y origen del plástico. Según la Enciclopedia Británica (https://www.britannica.com/science/plastic) el plástico es un "*material polimérico que tiene la capacidad de ser moldeado o conformado, habitualmente mediante la aplicación de calor y presión*". La presente introducción tratará de contextualizar el problema existente producido por la acumulación de plásticos y *microplásticos* en los mares y océanos, para lo cual es interesante saber desde cuándo el plástico forma parte de nuestra historia.

Pese a que el origen del primer plástico reconocido se atribuye a la bakelita, desarrollada por **Leo Hendrik Baekeland** en 1907 (Chalmin, 2019), el punto de partida en el desarrollo de este tipo de sustancias se remonta a principios del siglo XIX. Fue el químico francés **Henri Braconnot** (Commercy, Departamento de Mosa, Francia, 1780) quien, gracias a sus conocimientos en fisiología vegetal, abrió el camino para la posterior manufactura de productos sintéticos a partir de compuestos naturales. De entre sus descubrimientos, cabe destacar la pectina y la nitrocelulosa en 1833 (Wisniak, 2007). En el momento de este hallazgo, la naturaleza y el potencial uso de la nitrocelulosa no fue del todo entendido por Braconnot, por lo que sus investigaciones se paralizarían y no se recuperarían hasta pasadas varias décadas. En 1860 el metalúrgico e inventor británico **Alexander Parkes** produjo un compuesto plástico maleable y artificial en respuesta a la escasez de materiales de dichas características en la época: la parkesina (Mossman, 2017; Rasmussen, 2021), patentada en 1865. El gran recibimiento de esta sustancia supuso la fundación de la empresa *The Parkesine Company, Ltd* en Londres, en 1866. El origen de la parkesina marcó un antes y un después en la historia de los polímeros artificiales conocidos oficialmente como "plásticos", por lo que Parkes gozó de gran reconocimiento en la época (Figura 1.1).

Figura 1.1: Placa conmemorativa en honor de Alexander Parkes, erigida por la sociedad cívica de Birmingham en 2004 en el lugar donde se alzaba la Elkington, Mason & Company, Electroplaters (1836 – 1868), lugar donde trabajó.

La revolución derivada de la parkesina motivó a numerosos inventores, de entre los que cabe destacar a los hermanos **Hyatt**. En 1868, y movidos por un concurso organizado en una empresa de bolas de billar, estos hermanos retoman las investigaciones de Braconnot, y producen nitrocelulosa estable y funcional modificada mediante alcanfor como agente plastificante. De esta manera, las bolas de billar de nitrocelulosa sustituirían a las habituales bolas de la época, fabricadas en marfil de incisivo de elefante (Chalmin, 2019). Este compuesto recibió el nombre de *celuloide*, lo que llevó a la creación de la *Celluloid Manufacturing Company*¸ en Nueva York (Hyatt, 1914; Seymour & Kauffman., 1992; White, 1999). Tras 40 años de dominancia del celuloide y de la parkesina, el químico belga-estadounidense **Leo Hendrik Baekeland** sintetizó, a partir de reacciones entre cuerpos fenólicos y formaldehidos, un compuesto denominado *bakelita* (o baquelita). Esta bakelita se reconoce, a día de hoy, como el primer compuesto plástico totalmente artificial (Baekeland, 1909), cuyo uso se extiende hasta nuestros días como aislante térmico. Con el paso de las décadas, estas resinas derivadas de formaldehidos fueron cayendo en desuso. Durante las primeras tres décadas del siglo XX, los materiales plásticos (que no plásticos como tal) disponibles eran la goma laca (resina natural utilizada en Europa desde la Edad Media, atribuida a los antiguos pueblos de la India), la <u>gutapercha</u> (trans-polímero del poli-isopreno o caucho natural originaria del sudeste asiático), la <u>ebonita</u> (goma natural calentada con elevadas cantidades de sulfuros, manufacturada paralelamente en EE.UU. y en Gran Bretaña en 1851) y el <u>celuloide</u> de los hermanos Hyatt (Gilbert, 2017). En la década de 1930, comenzarían a ver la luz los polímeros plásticos utilizados y distribuidos a nivel global hoy en día.

El siglo XX se caracterizó por numerosos eventos clave en la historia, muchos de ellos guerras. Estos eventos bélicos ejercieron como motor de investigación y producción, debido a la creciente demanda de compuestos resistentes, maleables, ligeros y duraderos. Con ello surgieron los polímeros plásticos que se utilizan todavía de manera habitual y de los que actualmente somos dependientes. Estos compuestos, clasificados en siete grupos en función de su reciclabilidad, siendo el número 1 muy sencillo de reciclar y el 7 el más complicado, se presentan en la Figura 1.2.

Figura 1.2: Clasificación de los plásticos modernos según su reciclabilidad.

2. Los plásticos en la actualidad

2.1. Visión global

Una vez contextualizado históricamente el origen de los plásticos, parece necesario indicar que los plásticos tienen una serie de ventajas que se podrían resumir en manufactura económica, ligereza, maleabilidad, gran resistencia (química, física y térmica), conductividad eléctrica nula, así como propiedades ópticas de interés (transparencia, índices de refracción elevados) y una elevada reciclabilidad en varios de ellos (Van der Vegt, 2006). La realidad es que, atendiendo a la percepción general, los plásticos son vistos como compuestos de gran utilidad para las actividades cotidianas Aliados que hacen de las tareas y acciones habituales algo más simple o fácil.

Por otro lado, el valor económico derivado de la industria del plástico ha de ser reconocido. La asociación PlasticsEurope publica anualmente el reporte anual del sector de manufactura del plástico en Europa; en el año 2021 (PlasticsEurope, 2022), este sector registró cerca de 50.000 empresas y dio trabajo a aproximadamente 1,5 millones de trabajadores, con una facturación cercana a los 400.000 millones de euros. Estos valores se alcanzaron como consecuencia de un incremento en la producción de plástico durante la pandemia del COVID-19, en 2020. A nivel mundial, la producción de plástico se ha visto incrementada en los últimos años (Figura 1.3). De entre las 390,7 Mt de plástico producidas a nivel mundial en el año 2021, el 15% han sido manufacturadas en Europa, lo que supone una cantidad en torno a las 58,6 Mt de plástico. Asimismo, del plástico producido en Europa en el año anterior (2020) únicamente se reciclaron cerca de 10 Mt, por lo que el balance neto de producción de plástico en Europa en el año 2021 fue de, aproximadamente, 48,6 Mt con respecto al año 2020 (PlasticsEurope, 2021).

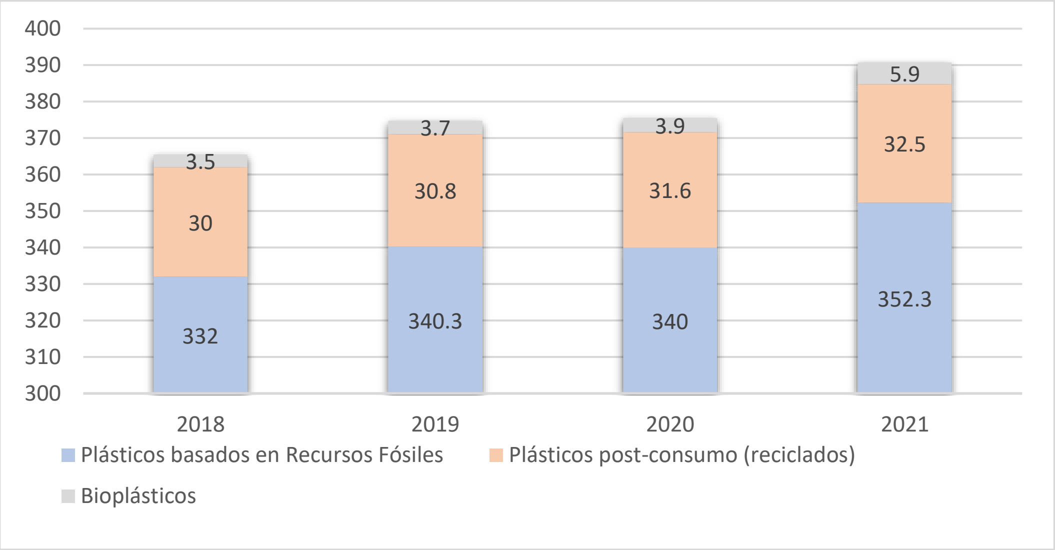

Figura 1.3: Megatoneladas de plástico producidas a nivel mundial entre los años 2018 y 2021. Fuente: PlasticsEurope, 2022.

Aunque no todo el plástico se convierte en desecho hasta transcurrido un largo periodo de tiempo, como puede ocurrir con los electrodomésticos, vehículos o edificaciones, millones de productos de plástico son de un solo uso. El ciclo de gestión del plástico (resumido en la Figura 1.4), pese a no ser complejo, presenta diversos puntos clave para tener en cuenta. Algunos de estos puntos pueden ser el reciclado de residuos sólidos, la acumulación de plásticos en cuencas hidrográficas o su depósito y acumulación finales en el océano, objeto de la presente Tesis.

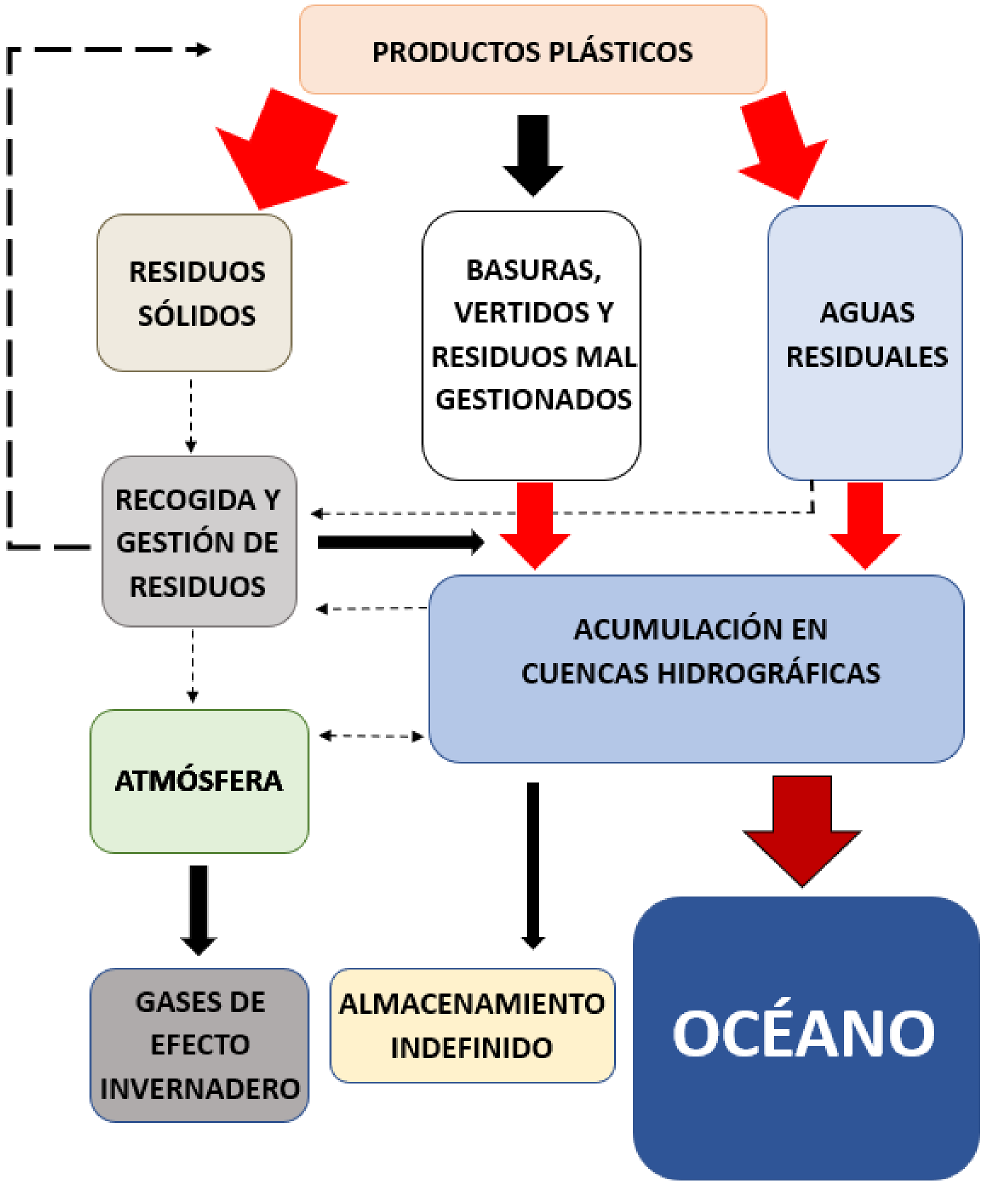

Figura 1.4: Ciclo de gestión del plástico. Modificado de Hoellein & Rochman, 2021.

En 1972, Carpenter y Smith reportaron por primera vez, en el Mar de los Sargazos, la presencia de abundante plástico en el medio marino, que no ha parado de crecer hasta nuestros días. Según recoge el Programa Medioambiental de las Naciones Unidas (UNEP, https://www.unep.org/plastic-pollution), "*Cada minuto se vierte a nuestros océanos el equivalente a un camión de basura de plástico*". Asimismo, indica que, de entre las incontables toneladas de plástico producido a nivel global entre los años 50 y el año 2017, se estima que entre 7 y 9,5 miles de millones de toneladas se convirtieron en residuos. Solamente entre 2010 y 2017 se estima una acumulación de entre 28 y 71 millones de toneladas de nuevos plásticos en mares y océanos (Beaumont et al., 2019). Las predicciones indican que la cantidad actual de plástico acumulada por los ambientes marinos se triplicará en el año 2040 (https://www.nature.com/articles/d41586-022-03835-w).

Pero ¿cómo llegan esos plásticos al mar y cómo se acumulan? Ya en 1987, Pruter identificó cuatro puntos principales de entrada de los plásticos al ambiente marino: basura generada en barcos (Jones, 1995; Thushari & Senevirathna, 2020; Bergmann et al., 2022) (Figura 1.5a), basura movilizada hacia el mar a través de los ríos (Castro-Jiménez et al., 2019; Helinski et al., 2021; Mitchel et al., 2021) (Figura 1.5b), sistemas municipales de alcantarillado (Mrowiek, 2017; Altuğ & Erdoğan, 2022; Çevik et al., 2022) (Figura 1.5c) y basura depositada/olvidada por usuarios de playas y zonas costeras (Figura 1.5d).

Figura 1.5: A) Tortuga marina enredada en una red de pesca (https://www.nationalgeographic.com.es/. Fotografía de Jordi Chías). B) Río sobrecargado de basura marina (https://www.nationalgeographic.com.es/. Fotografía de Rafael Neddermeyer). C) Planta de tratamiento de aguas (EDAR o WWTP) (https://commons.wikimedia.org. Fotografía de Annabel). D) Jeringa sobre la arena, Nida Baltic, Lituania (Fotografía de D. Menéndez).

Las actividades antrópicas en regiones costeras se encuentran reguladas y el depósito incontrolado de plásticos es sancionado según recoge la Ley 22/2011, del 28 de Julio, de residuos y suelos contaminados. En el año 2022, se reformuló con la Ley 7/2022, del 8 de abril, de residuos y suelos contaminados para una economía circular, donde los mares y océanos pasaron a ser reconocidos como compartimentos amenazados medioambientalmente y, por ende, protegidos. Respecto al origen principal de esta basura plástica, Pawar y col. (2016), clasifican las fuentes de plástico en dos grandes grupos: fuentes terrestres (80%) y marinas (20%) (Tabla 1.1).

Tabla 1.1: Origen de la basura marina. Información extraída de Pawar et al. (2016).

Tipo de fuente	**Origen de la basura marina**	**Modo de llegada al mar/océano**	**Anotaciones**
Terrestre	*Descargas de agua de tormenta*	Depósito directo en el medio marino o llegada a través de los ríos	
	Desbordamiento del alcantarillado	Depósito directo en el medio marino o llegada a través de los ríos	Efecto combinado con "Descarga de aguas de tormenta"
	Depósito de basura directo	Depósito directo en playas, zonas costeras o ríos	La basura incluye empaquetado de comida y bebida, cigarros y juguetes de playa. También incluye la deposición de redes de pesca por parte de pescadores, así como basura producida a lo largo de potenciales ríos
	Sistemas de gestión de basuras y vertederos	"Escapes" inintencionados	Incluye vertidos ilegales de basuras industriales y domésticas
	Actividades Industriales	Gestión deficiente de residuos o vertido accidental en puertos	Microesferas y pellets de plástico son liberadas en el proceso de transporte y manejo de mercancías
Marina	*Pesca comercial*	Error al recoger las redes de pesca o su descarte directo en el medio	Incluye redes, sedal, cabos, flejes, carnada, cajas, bolsas o flotadores de arrastre
	Barcos de recreo	Disposición directa por parte de los usuarios	Bolsas, empaquetado de comidas y bebidas o redes de pesca
	Vehículos mercantes, militares y de investigación	Vertido accidental o deliberado por falta de espacio	
	Plataformas petrolíferas y de prospección de gases	Vertido accidental o deliberado por falta de espacio	Cascos de seguridad, guantes, cajas de almacenamiento o deshechos de uso personal

Sea cual sea su origen, una vez los plásticos han alcanzado el medio marino la problemática se amplía debido a su distribución (van Sebille et al., 2020), tanto vertical (eje fondo-superficie) como horizontal. La primera viene determinada por tres factores principales (Schwarz et al., 2019): densidad del polímero, área superficial del polímero y tamaño de las partículas o fragmentos. Según Pattiaretchi y col. (2022), en torno al 35% de los plásticos son más densos que el agua del mar, lo que conlleva su hundimiento y su depósito en los fondos marinos. El resto, son menos densos que el agua, con una distribución flotante (Cadeé, 2002; van Sebille et al., 2015), o en suspensión en la columna de agua (Corcoran, 2015; Egger et al., 2020) que conlleva su desplazamiento horizontal influenciado por los vientos y las corrientes marinas (Kershaw et al., 2011). Los giros oceánicos (gran sistema de corrientes oceánicas rotatorias- https://oceanservice.noaa.gov/facts/gyre.html) favorecen la acumulación del plástico flotante movilizado por las corrientes en las denominadas "Islas de Basura" o *Garbage patches* en inglés. Acorde a la información proporcionada por la iniciativa *"The Ocean Cleanup"* (https://theoceancleanup.com/), dedicada a la eliminación de las grandes islas de plástico y del saneamiento de ríos muy contaminados, en la actualidad se ha documentado la existencia de cinco grandes islas de basura, una por cada gran giro oceánico (Figura 1.6). Destacando la isla de plástico del Pacífico norte (número 1 en el mapa), con una extensión en superficie estimada en torno a 1,6 millones de Km² (equivalente a la extensión de Irán o tres veces la superficie de España) y 80.000 toneladas de basura flotante (Greenly et al., 2021).

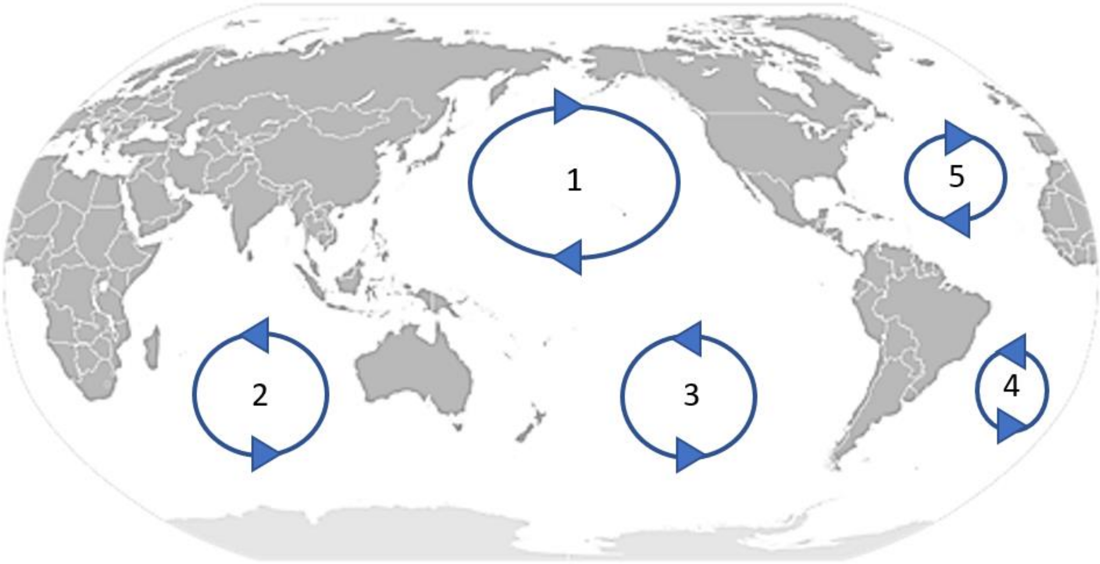

Figura 1.6: Localización de las cinco grandes islas de basura. 1 - Pacífico Norte, 2 - Índico, 3 - Pacífico Sur, 4 - Atlántico Sur y 5 - Atlántico Norte. Extraído de https://theoceancleanup.com/.

La identificación y estudio detallado de las fuentes contaminantes en mares y océanos permitirá la mejora y perfeccionamiento de los sistemas de prevención, los cuales funcionan como "escudo" frente a la llegada de plástico a los ambientes acuáticos. Lo que nos lleva al objeto de estudio de esta Tesis, los microplásticos (MPs en adelante).

3. Microplásticos

3.1. Descripción y clasificación

Los MPs son plásticos de pequeño tamaño, de entre 1 µm y de 5 mm (Andrady, 2011; Gigault et al., 2018), lo que los hace prácticamente invisibles al ojo humano y los convierte en un peligro añadido para el ecosistema y los organismos que en él habitan, incluido el ser humano. ¿Cómo se produce un plástico de tamaño tan reducido? los MPs se pueden producir ya sea directamente de ese tamaño o como resultado de la actuación de factores ambientales físicos y químicos sobre plásticos más grandes, siendo denominados MPs primarios y MPs secundarios respectivamente.

- **MPs primarios:** Aquellos de manufactura directa con ese tamaño. Se emplean en productos de higiene personal como pasta de dientes, maquillajes o toallitas desmaquillantes. Por otro lado, también son MPs primarios los producidos en la industria por abrasión de plásticos mayores, los derivados de utilización directa como el desgaste de neumáticos o incluso la liberación de microfibras durante el lavado de prendas textiles (Boucher & Friot, 2017; Wang et al., 2019; Dissanayake et al., 2022).

- **MPs secundarios:** En este caso surgen de la fragmentación y/o degradación *in situ* de plásticos de mayor tamaño. Su aparición responde al efecto directo de factores ambientales bióticos y abióticos, tales como la fragmentación por parte de organismos en su búsqueda de alimento, la incidencia de radiación UV y viento, e incluso el efecto movilizador y deformante de las corrientes (Efimova et al., 2018; Jaikumar et al., 2019). Debido a la gran cantidad de macroplásticos que llegan al océano cada día, se estima que los MPs secundarios son, con mucha diferencia, los más abundantes en el medio marino (Duis & Coors, 2016).

Otro tipo de clasificación habitual atribuida a los MPs es basada en su morfología. La mayoría de los investigadores reconocen comúnmente tres formas diferentes (Figura 1.7): fibras, fragmentos y microesferas o pellets (Hidalgo-Ruz et al., 2012; Ngo et al., 2019; Lorenzo-Navarro et al., 2021).

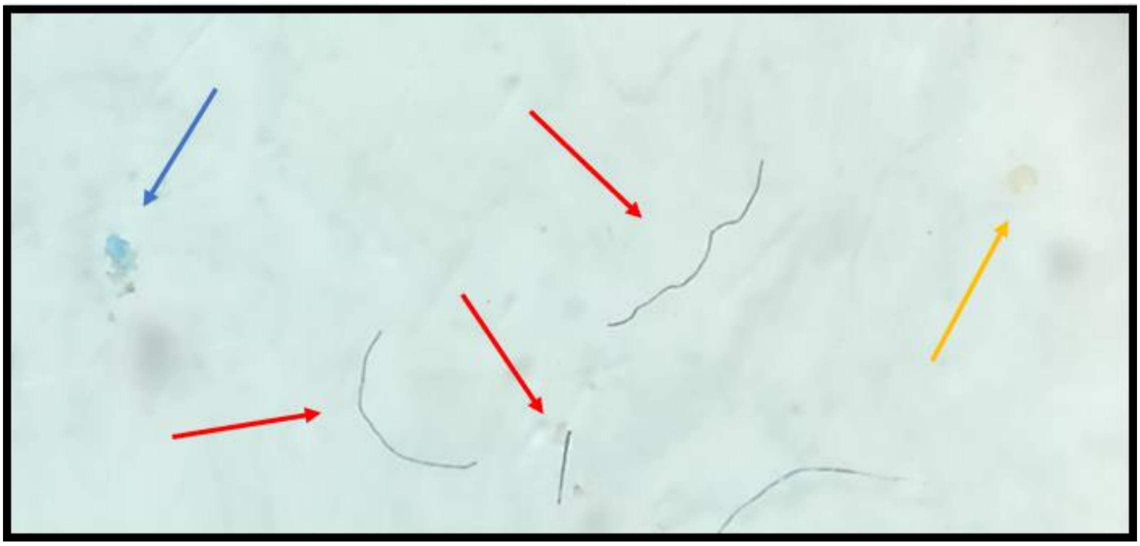

Figura 1.72: Visualización de microplásticos al microscopio óptico con 40x aumentos. Se señalan los tres tipos de morfologías descritas habitualmente para los MPs, preparadas para su análisis químico durante el desarrollo de esta Tesis. Flecha azul: fragmento. Flechas rojas: fibras. Flecha naranja: microesfera. Fotografía por D. Menéndez.

3.2. Distribución de los MPs en el medio ambiente

Miles de estudios avalan la presencia ubicua de MPs en todos los ambientes conocidos. Desde la nieve ártica (Bergmann et al., 2019), hasta el desierto profundo (Abbasi et al., 2021), pasando por grandes altitudes (Allen et al., 2021), profundidades abisales (Jamieson et al., 2019) y hasta miles de kilómetros mar adentro (Lebreton et al., 2018). Pero esta ubicuidad no sucede exclusivamente en el medio, sino que también está reflejada en los organismos vivos. Independientemente del nivel trófico, todas las especies vivas son susceptibles de ser afectadas en una medida u otra por MPs. Tanto en su interior como en su superficie, numerosos estudios han indicado presencia y abundancia de MPs en un amplio abanico de organismos, desde algas (Gutow et al., 2016) y vegetales terrestres (Austen et al., 2022) hasta grandes cetáceos (Zhu et al., 2019; Moore et al., 2020), pasando por plancton (Zhang et al., 2017; Lima et al., 2015), moluscos (Masiá et al., 2022) y aves (Masiá et al. 2019). Recientemente se ha publicado la presencia de MPs dentro del cuerpo humano, tanto en la placenta (Ragusa et al., 2021), como en la sangre (Leslie et al., 2022).

En el medio marino, se han encontrado MPs en distintos ecosistemas y a diferentes profundidades (estudios en arena de playa y en aguas de superficie, así como en la columna de agua y en el fondo oceánico) (Castillo et al., 2016; Qu et al., 2022). Actualmente se reconoce que los MPs se encuentran presentes en todos los compartimentos acuáticos de nuestro planeta, tanto en ríos y lagos (Driedger et al., 2015; Blettler et al., 2018; Van Emmerick & Schwarz, 2019; Sheridan et al., 2022), pasando por glaciares (Ambrosini et al., 2019; Allen et al., 2021; González-Pleiter et al., 2021) como en mares y océanos (Cózar et al., 2014; Masiá et al., 2019; Cabanilles et al., 2022;

Menéndez et al., 2022; Menéndez et al., 2023) y aguas subterráneas (Viaroli et al., 2022), siendo un campo de investigación en continuo crecimiento. El estudio de los MPs en el medio marino ha ido ganando importancia de manera exponencial a lo largo de las últimas décadas. En 2004 se empezó a conocer a los fragmentos de plástico menores de 5 mm como MPs, siguiendo a Thompson (2004). La revisión llevada a cabo por Zhou y col. (2022), muestra cómo entre el año 2004 y el 2010 se iniciaba el estudio de los MPs marinos, con 70 artículos publicados. Tras este periodo, entre los años 2011 y 2017, el número de artículos publicados del mismo ámbito ascendió hasta 939, mientras que en los últimos tres años estudiados (2018-2020), el número subió a 2.335 publicaciones. Esto pone de manifiesto un claro interés científico por conocer la presencia, distribución y efectos de los MPs en el medio marino.

De entre los 3.344 artículos publicados hasta el año 2020, son pocos los que han intentado estimar la abundancia total de MPs en mares y océanos a nivel global. Debido a su reducido tamaño, calcular la cantidad total de MPs en todos los ambientes marinos del planeta es actualmente imposible. No obstante, varios grupos han tratado de llevar a cabo estimaciones basadas en muestreos masivos que han permitido tener una idea aproximada. Según estimaron Isobe y col. (2021) y Barret y col. (2020), se encuentran en torno a 24,4 trillones y 14,4 trillones de toneladas de partículas identificadas como MPs en las aguas pelágicas y en las capas más superficiales de los sedimentos oceánicos, respectivamente.

Las vías de entrada de los MPs al ambiente marino son las mismas que para los plásticos de mayor tamaño. Como fuentes de MPs de difícil cuantificación y estimación, cabe destacar el abandono de plásticos y derivados en playas (Bissen & Chawchai, 2020) y zonas de pesca (Dowarah & Depriya, 2019). Otra vía de contaminación importante en alta mar son los barcos mercantes, de recreo y de pesca (Aretoulaki et al., 2021; Turner, 2021). Los ríos actúan, como se ha visto previamente, como fuentes constantes de MPs hacia el medio marino (Siegfried et al., 2017; Xiong et al., 2019). Por otra parte, la presencia de las plantas de tratamiento de agua o EDARs (Estación Depuradora de Aguas Residuales), encargadas de eliminar contaminantes de aguas urbanas e industriales mediante diversos procesos químicos y físicos (Hreiz et al., 2015), a menudo no se encuentran diseñadas para la retención específica de este tipo de contaminantes. Su eficacia de retención se sitúa actualmente en torno al 90% de los MPs entrantes (Murphy et al., 2016; Masiá et al., 2019). El 10% restante, junto con los MPs que llegan directamente de pequeños ríos y que se acumulan a lo largo de su recorrido, supone un aporte constante e importante de estos contaminantes al mar (Talvitie et al., 2015).

Las aguas subterráneas, al igual que los ríos, recogen MPs acumulados en zonas de tierra firme. Esta acumulación se produce por filtración a través de las capas de suelo durante lluvias, regadíos o actividades industriales (Re, 2019; Su et al., 2021; Khant & Kim, 2022). Estas aguas, según la UNESCO, representan "el 98% del agua dulce no congelada" (https://es.unesco.org/themes/garantizar-suministro-agua/hidrologia/agua-subterranea), y son utilizadas por el ser humano para abastecerse en las actividades diarias.

La última fuente de contaminación marina merecedora de mención en este punto, y cuya investigación ha ganado relativa importancia durante los últimos años, es la contaminación aérea. Es decir, la movilización de MPs desde zonas contaminadas (generalmente urbanas) hasta localizaciones remotas sobre la superficie de mares y océanos (Liu et al., 2019; Trainic et al., 2020). Finalmente, estos MPs precipitan y se incluyen en el eje vertical superficie – columna de agua – fondo marino (Enyoh et al., 2019), reincorporándose nuevamente a la atmósfera desde la superficie del agua (Ferrero et al., 2022).

3.3. El (micro)plástico como contaminante marino: de micropartículas a macroproblemas

El creciente interés y demanda de los plásticos ha conllevado su producción masiva. Desgraciadamente, estos compuestos que parecían surgir como una solución rápida y duradera a multitud de problemas cotidianos se han ganado la etiqueta de contaminantes emergentes. Hoy en día, los plásticos son reconocidos a nivel mundial como un problema medioambiental de grandes dimensiones. Así lo recoge la UNEP en la Asamblea del Programa Medioambiental de las Naciones Unidas, celebrada en Nairobi, Kenia, en 2022. Al comienzo de su resolución, se reconoce la contaminación por plásticos como "*Un problema medioambiental serio en una escala global, que impacta negativamente en los aspectos medioambientales, sociales y económicos del desarrollo sostenible*" (UNEP/EA.5/Res.14). El problema no reside únicamente en el plástico como elemento en sí mismo, sino que el proceso de producción, transporte y gestión de dichos compuestos es una fuente activa de producción de gases de efecto invernadero (Shen et al., 2020), estrechamente relacionados con el calentamiento global (Kweku et al., 2018).

Según la revisión publicada por Worm y col.s en 2017, Gal & Thompson (2015) estimaron que, en el año 2015, había 693 especies afectadas por basura marina. Recientemente, un reporte del Alfred Wegener Institute (Tekman et al., 2022), basado en una exhaustiva revisión bibliográfica, cifra esas especies en 2.788. De entre los diferentes tipos de interacción, los más significativos, ordenados en función del número de especies afectadas son: colonización, ingesta y enredo. La colonización de los plásticos por parte de los organismos vivos puede suponer, y así se encuentra ampliamente documentada, la dispersión de especies no nativas adheridas a su superficie (Miralles et al., 2018; Silva et al., 2019; García-Gómez et al., 2021; Fernández et al., 2022), con efectos negativos sobre el ecosistema. En el caso de los MPs y, limitado por la superficie a colonizar, los organismos movilizados son, mayoritariamente, microorganismos, tanto bacterianos (Stenger et al., 2021), como víricos (Moresco et al., 2022), fúngicos (Yang et al., 2020), y algas (Dudek et al., 2020).

En el caso de los MPs, todos los organismos, incluyendo vegetales, detritívoros, herbívoros, depredadores y filtradores (Hall et al., 2015; Lusher et al., 2017; Li et al., 2021) son susceptibles de ingerirlos o acumularlos en sus tejidos (Su et al., 2019; Bilbao-Kareaga et al., 2023), introduciéndolos en la cadena trófica (Carbery et al., 2018; Athey et al., 2020). La ingesta de plásticos produce efectos adversos sobre el organismo, al ser confundido con alimento (Mascarenhas et al., 2004; De-la-Torre, 2019; Clause et al., 2021). Esta ingesta expone los tejidos blandos del organismo a un cuerpo extraño. En varias ocasiones, se ha evidenciado la rotura de estos tejidos y la posterior transferencia de MPs a otros (Zhang et al., 2020; Chen et al., 2022). De la misma manera que ocurre con los plásticos de gran tamaño, el organismo afectado puede percibir la sensación de falsa saciedad (Hamid et al., 2020; Zhang et al., 2022), lo que puede incluso conllevar su muerte por carencias nutricionales.

Por otra parte, los MPs también tienen efectos a nivel molecular en los organismos que los ingieren y/o acumulan. El primero de estos efectos se deriva del proceso de manufactura de los materiales plásticos debido a los aditivos. Compuestos como los ftalatos, los bisfenoles (A, S, F), difenil éteres polibromados o el tetrabromobisfenol A (TBBPA), son empleados en el proceso de producción de una amplia variedad de compuestos plásticos (Basak et al., 2020), desde el PVC de las tuberías (Grupo 3) hasta el policarbonato empleado en biberones (Grupo 7). Estos productos han sido identificados como disruptores endocrinos, definidos como compuesto químico capaz de alterar el correcto funcionamiento del sistema endocrino de un organismo vivo (https://echa.europa.eu/es/understanding-ed-assessment). Esta modificación puede conllevar el desarrollo de patologías tales como alteraciones reproductivas, malfuncionamiento hepático o incluso el desarrollo de cáncer (Pupo et al., 2012; Basak et al., 2020).

Una de las características derivadas de la estructura química de los polímeros plásticos es la hidrofobicidad. De esta manera, la superficie de los plásticos conforma un ambiente propicio para la adsorción de compuestos nocivos dispersados por el medio, comúnmente conocidos como contaminantes orgánicos persistentes o POPs (Brennecke et al., 2016; Barboza et al, 2018; Ziani et al., 2023). Es decir, elementos perdurables y de difícil recuperación y gestión, una vez han entrado en el medio ambiente. La naturaleza de estos compuestos es muy variada, ya que, dentro de los POPs, se incluyen pesticidas, químicos industriales, hidrocarbonos aromáticos y metales pesados (Ighalo et al., 2022). Estos POPs, acumulados en grandes cantidades, pueden tener efectos adversos en múltiples niveles y tejidos, produciendo reacciones agudas o crónicas. Los efectos varían notablemente dependiendo del compuesto al que el organismo es expuesto. Algunos de estos efectos son daño en el sistema nervioso, pulmones, huesos o sistema cardiovascular, así como daño en riñones, piel o sistema reproductivo (González-Mille et al., 2010; Chen et al., 2019; Girones et al., 2021). De la misma manera, la exposición a metales pesados ha reportado distintos tipos de afección, tales como daño al ADN (Hengstler et al., 2003), daño enzimático (Prata et al., 2022) o producción de radicales libres descontrolados (Shengchen et al., 2021), los cuales se traducen en el desarrollo de estrés oxidativo intracelular (Rajkumar & Gupta, 2020). Igualmente, la exposición directa a plásticos a nivel celular se ha señalado como productora de patologías tales como daño intestinal (Duan et al., 2022), apoptosis celular (An et al., 2021), daño directo en el DNA (Masiá et al., 2021), modificaciones en la permeabilidad celular (Hirt & Body-Malapel, 2020) o inflamación y desórdenes metabólicos severos (Qiao et al., 2019).

El problema causado por los MPs puede alcanzar una magnitud mayor, más allá del organismo que ingiere/adquiere esos MPs. Se ha evidencias en multitud de ocasiones un proceso de transferencia entre niveles tróficos de MPs (Nelms et al., 2018; Tanaka et al., 2018; Athey et al., 2020), los cuales pasan de presas a depredadores. Actualmente no hay un consenso general en relación con una posible bioacumulación de MPs con respecto a niveles tróficos inferiores (Bhatt et al., 2023), pero se están llevando a cabo numerosas investigaciones (Akhbarizadeh et al., 2019; Walkinshaw et al., 2020 Qaiser et al., 2023) para saber si este proceso existe de manera natural o no. Para esto, es de vital importancia el estudio de MPs en organismos de diferente nivel trófico, comenzando por algas (productoras primarias, sésiles, afectadas directamente por los plásticos de la columna de agua -Bilgao-Kareaga et al., 2023) y terminando con depredadores de la cima de la cadena trófica (móviles, acumulación activa de MPs, adquisición de MPs de sus presas directas – Janardhanam et al., 2022).

Pese a los numerosos estudios realizados sobre el impacto de MPs sobre organismos marinos, en el momento de comenzar esta Tesis había numerosas cuestiones que no se habían tratado aún. Entre ellas destacaban los impactos y consecuencias de la contaminación por MPs sobre las algas, mucho menos estudiadas que los animales. Aunque se sabía que las algas retienen MPs (Esiukova et al., 2021), no se habían explorado los mecanismos que podrían incrementar o mitigar la cantidad de MPs en derivados de algas ampliamente consumidos como el agar. Otra cuestión abierta era el rol de los organismos migratorios en el transporte de MPs; si bien se ha investigado este aspecto parcialmente en especies mesopelágicas con migración vertical (Justino et al., 2022), o en grandes peces marinos migratorios como los atunes (Pereira et al., 2023), en la mayoría de las especies diádromas que migran entre el río y el mar en distintas etapas vitales aún era un enigma al comienzo de esta Tesis. Finalmente, impactos físicos de los MPs como la degradación del ADN habían sido explorados solamente en invertebrados (Alnajar et al., 2021; Masiá et al., 2021). Por tanto, para conocer mejor la extensión de este fenómeno era necesario el estudio en otros organismos marinos, especialmente en vertebrados.

Junto con estos tres aspectos introducidos anteriormente, la escasez de información publicada sobre el contenido de MPs en agua y especies explotadas de la costa asturiana, y los pocos estudios que ponen de manifiesto este problema en las aguas del noroeste de África, con muy poca investigación sobre esta problemática en la zona, serán el objetivo de estudio de esta presente Tesis.

Objetivos

La presente Tesis tiene como objetivo general el análisis y estudio de los MPs adquiridos y movilizados por distintas especies de elevado interés comercial en la costa asturiana y las aguas del noroeste de África. Para ello, se analizan especies pertenecientes a diferentes niveles tróficos, con diferente estatus ecológico. Este objetivo general se llevará a cabo mediante el desarrollo de los siguientes objetivos parciales:

1. Exploración bibliográfica de la presencia, magnificación y control de contaminación en algas rojas gelificantes de interés comercial, y estudio de su ciclo de producción para identificar los puntos clave en los que se incorporan microplásticos y que deberían ser objeto de control.

2. Análisis de microplásticos en una especie catádroma en peligro crítico de extinción, la anguila europea (*Anguilla anguilla*), a su llegada a los ríos de la costa asturiana, valorando las diferentes vías de acceso a estos contaminantes y el posible proceso de bioacumulación.

3. Cuantificación de microplásticos en merluza negra o merluza de Benguela (*Merluccius polli*) pescada en el Atlántico central (costa noroeste de África), y valoración del daño fisiológico y la degradación del ADN en individuos con distinto grado de contaminación.

4. Basándose en los resultados obtenidos en los apartados anteriores, realizar un análisis general de riesgos asociados a la presencia de MPs, tanto para el ecosistema como para el consumidor final de las especies analizadas, así como recomendaciones orientadas a la eliminación o al menos la mitigación de estos contaminantes en el medio marino.

Objectives

The present thesis has as general objective the analysis and study of the MPs acquired and mobilized by different species of high commercial interest in the Asturian coast and the waters of northwest Africa. For this, species belonging to different trophic levels, with different ecological status, are analyzed. This general objective will be carried out through the development of the following partial objectives:

1. Bibliographic exploration of the presence, magnification and control of contamination in red gelling algae of commercial interest, and study of their production cycle to identify the key points in which microplastics are incorporated and that should be controlled.
2. Analysis of microplastics in a catadromous species critically endangered of extinction, the European eel (*Anguilla anguilla*), upon its arrival in the rivers of the Asturian coast, assessing the different access routes to these contaminants and the possible bioaccumulation process.
3. Quantification of microplastics in the Black hake or Benguela hake (*Merluccius polli*) fished in the central Atlantic (northwest coast of Africa), and assessment of physiological damage and DNA degradation in individuals with different degrees of contamination.
4. Based on the results obtained in the previous sections, carry out a general analysis of the risks associated with the presence of MPs, both for the ecosystem and for the final consumer of the analyzed species, as well as recommendations aimed at eliminating or at least eliminating them. mitigation of these pollutants in the marine environment.

Materiales y Métodos

1. Áreas de estudio: costa asturiana y aguas africanas

La presente Tesis se enmarca principalmente en el mar Cantábrico, con un estudio de caso en aguas del Noroeste de África.

1.1. COSTA ASTURIANA.

El mar Cantábrico, situado en el Golfo de Vizcaya, baña la costa norte de España y la costa suroeste de Francia, desde el Cabo Ortegal, en A Coruña, hasta la desembocadura del río Adour, en Bayona. Comprende aproximadamente 800 Km de paisaje costero escarpado (500 Km en línea recta), caracterizado por sus marcados acantilados (Domínguez-Cuesta et al., 2019) y por la desembocadura de numerosos ríos (unas 34 cuencas hidrográficas- Masiá et al., 2021). La presente Tesis se desarrolla en la costa asturiana, de 401 Km de extensión (https://www.ign.es/web/ane-datos-geograficos/-/datos_geograficos/datosGenerales?tipoBusqueda=longCosta). Hasta la fecha, esta es la segunda Tesis doctoral dirigida al estudio de MPs en recursos marinos de interés comercial explotados en esta región, así como del Golfo de Vizcaya.

Según el Instituto Nacional de Estadística, en el año 2022, 470.184 habitantes se encontraban censados en los municipios costeros asturianos, un 46.8% del total de la población asturiana, 1.004.686 habitantes). Esto refleja la importancia de las actividades costeras y marítimas en la economía de la comunidad autónoma. Asimismo, esta concentración de la población puede conllevar la producción masiva de desechos plásticos y su vertido en el mar.

El mar Cantábrico está recorrido por la corriente del Atlántico Norte (Marquina et al., 2015), que moviliza la masa de agua en dirección este (Flor & Flor-Blanco, 2005). Estas corrientes, cuando discurren cercanas a la costa, se encuentran con el accidente geográfico del Cabo Peñas (Domínguez-Cuesta et al., 2019). Este, separa dos regiones claramente diferenciadas en función de su productividad para la industria pesquera y el estrato sedimentario del fondo marino: una oriental menos productiva, de fondos fangosos, y una occidental, más productiva de fondos arenosos (Serrano et al., 2011).

En esta Tesis, se han estudiado las siguientes zonas de la costa asturiana:

1. **Desembocadura de los ríos Nalón, Cabra y Bedón**: Estudios desarrollados en la interfaz agua salada - agua dulce. Primer estudio en profundidad de una especie cátadroma (nacimiento en el mar y crecimiento en agua dulce) en peligro crítico de extinción (Anguila europea – *Anguilla anguilla*) en su relación con los MPs y su actuación como vectores entre compartimentos de la biosfera. Este organismo es

considerado de elevado interés comercial, pero por su estado crítico de conservación hay limitaciones en su pesca (cuatro meses al año y solamente juveniles, llamados angulas en Asturias).

2. **Costa Asturiana en extensión. Mar adentro:** Adquisición de muestras de agua de mar en localizaciones situadas frente a la costa asturiana. Se utilizaron para inferir la potencial fuente de adquisición de MPs por parte de la Anguila europea en contraposición a los MPs aportados por el río.

1.2. AGUAS AFRICANAS.

2.000 km al sur de la costa asturiana se encuentra la costa noroeste de África, en particular el área de pesca FAO 34.1.3. (https://www.fao.org/fishery/en/area/34/en), la cual comprende desde el Sáhara Occidental (26° N) hasta las costas del norte de Mauritania (19° N).

La zona costera que bordea el área FAO 34.1.3 tiene comunidades dedicadas a la pesca, con una menor densidad poblacional, debido principalmente a su naturaleza desértica. Esta área de estudio se encuentra afectada por corrientes en direccionalidad Norte–Sur, desde el golfo de Cádiz (https://earth.nullschool.net/#current/ocean/surface/currents/orthographic=-14.79,27.23,1849). A su vez, el Golfo de Cádiz es el punto de recepción de aguas intercambiadas entre el océano Atlántico y el Mar Mediterráneo (Ochoa & Bray, 1991) a través del Estrecho de Gibraltar, movilizando, así, contaminantes desde una región a otra (Laiz et al., 2020; Besada et al., 2022).

En la región estudiada del noroeste africano, los recursos pesqueros son de gran importancia para la flota española (Rey et al., 2012; Soto et al., 2022). Hasta la fecha, esta Tesis presenta una de las pocas evidencias publicadas sobre el efecto nocivo de los MPs en organismos demersales del Noroeste de África. La especie estudiada es la merluza negra o de Benguela (*Merluccius polli*), simpátrica de la merluza de Senegal (*Merluccius senegalensis*) en esta zona y cuya comercialización en España y Europa es de notable importancia económica, sobre todo para las lonjas del sur peninsular.

2. Materiales y metodologías utilizados

Las metodologías, así como los materiales empleados en cada investigación realizada en esta Tesis, se encuentran detallados en el apartado "*Materials and methods*" de cada uno de los capítulos que componen los resultados. A continuación, se exponen de manera sucinta a fin de generar una visión general, indicando en qué capítulos fue llevado a cabo cada proceso.

- ***Capítulo 1:*** Algas
- ***Capítulo 2:*** Anguilas
- ***Capítulo 3:*** Merluzas

1. Búsqueda bibliográfica

Se realizó mediante la utilización de bases de datos y repositorios científicos (PubMed & Google Académico). Comprobación de los artículos seleccionados como "revisados por pares" mediante la plataforma virtual de la Fundación Española de Ciencia y Tecnología (https://www.recursoscientificos.fecyt.es/servicios/). → ***Capítulo 1.***

Palabras Clave: *"Microplastics seaweed uptake", "Microplastics algae adherence", "Microplastics in agar/carrageenan producers", "Microplastics in Gelidium/Gracilaria", "Microplastics in gelling agents", "Pollution agar/carrageenan", "Industrial manufacturing pollution", "Microplastics adherence handling", "Microplastics in fisheries"* y *"WWTPs as sources of microplastics".*

2. Toma de muestras de agua y sedimento

Se recolectaron muestras de agua de mar en mar abierto, en la costa, y en la desembocadura de los ríos, y de sedimento en varios de estos puntos. El agua se almacenó en garrafas de 5L previamente enjuagadas con agua destilada filtrada para evitar una potencial contaminación cruzada durante el proceso de manipulación y transporte. Por otro lado, las muestras de sedimento se recogieron mediante botes de cristal, también enjuagados con agua destilada filtrada.

Esta recolección se llevó a cabo en dos áreas diferentes:

- En mar abierto: Se utilizó una garrafa de agua de 5L por punto de muestreo (aproximadamente 245 L en total- 49 muestras). Agua recolectada por pescadores de la flota asturiana → ***Capítulo 2***. Figura 1.1: Puntos verdes.
- En zonas de desembocadura de ríos: Se recogieron 4 garrafas de 5L por punto de muestreo (20L por punto, 60L en total). En las playas situadas en la desembocadura de dichos ríos, se recogieron 20 botes de 50 gr de sedimento (60 botes para un total de 3.000 g de sedimento) → ***Capítulo 2***. Figura 1.1: Estrellas rojas.

3. Muestras de organismos

Las muestras de angulas fueron cedidas al CEP (Centro de Experimentación Pesquera del Principado de Asturias) por las Cofradías de Pescadores de Asturias → ***Capítulo 2*** (Figura 1.1:

Estrellas rojas). Por otro lado, y atendiendo a la legislación vigente en materia de bioética, los organismos empleados en el ***Capítulo 3*** (Merluza de Benguela) son procedentes de pesquerías comerciales (pesca de arrastre), y fueron proporcionadas por la Lonja de Cádiz (Figura 1.1: Punto Blanco – Zona FAO 34.1.3.).

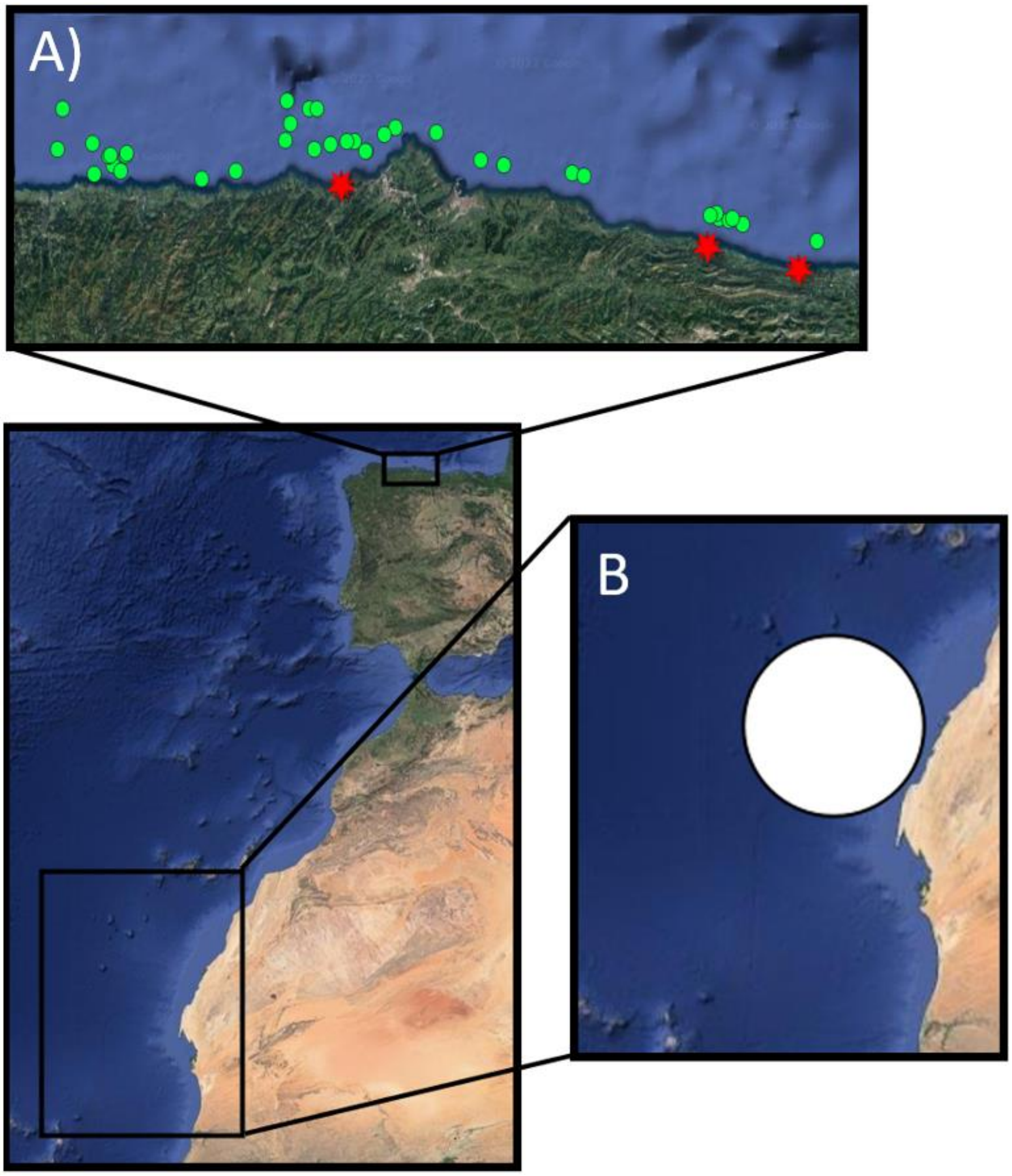

Figura 1.1.: Localización de los puntos de muestreo considerados durante el desarrollo de la presente Tesis. A) Costa asturiana. Se representan los puntos de muestreo de agua de mar (puntos verdes) y ríos de donde se obtuvieron muestras de angulas (estrellas rojas), B) Aguas del noroeste africano. Se representa el área de captura de la merluza de Benguela analizada en este trabajo (círculo blanco) Mapa realizado mediante QGIS v. 3.16.1

4. <u>Procesamiento de agua, sedimentos y organismos</u>

Las diferentes muestras se procesaron al llegar al laboratorio siguiendo principalmente los protocolos desarrollados por Masiá y col. (2022), en condiciones controladas en todo caso para evitar la contaminación por MPs del aire o transportados por los materiales en contacto con el material de interés:

- **Agua:** Las garrafas de agua fueron filtradas inmediatamente a su llegada al laboratorio, mediante vacío a través de filtros de PES (PolyEther Sulphone) con poro de 0.22 µm → ***Capítulo 2.***
- **Sedimento:** Se pusieron 50g de cada muestra en una solución de agua hipersalina filtrada (159,8 g de sal común por cada 1L de agua destilada). El proceso de flotación se extendió 24 horas, durante las cuales se agitaron manualmente para liberar las partículas retenidas en el sedimento. Una vez transcurrido el tiempo estipulado, la solución de agua salina con MPs fue filtrada a través de filtros de 0.45 µm de poro → ***Capítulo 2.***
- **Organismos:** El tejido de estudio se digirió por tiempo variable en un solvente de materia orgánica. Una vez transcurrido el tiempo de la digestión, la solución resultante fue filtrada a través de filtros de 0.45 µm de poro:
    - **Angulas:** Digeridas con peróxido de hidrógeno (H_2O_2) al 30% (w/v) durante una semana (168 horas), dentro de un horno a 65ºC a razón de 20 ml por gramo de tejido → ***Capítulo 2.***
    - **Merluzas:** En el caso de las merluzas, el reactivo elegido fue el hidróxido de potasio (KOH) diluido al 10% (w/v). Los distintos tejidos se digirieron con proporciones de solvente diferentes: El hígado fue digerido a razón de 50 ml por cada 1 gramo de tejido, el músculo a razón de 5:1 y las branquias, de 10:1. El proceso de digestión se llevó a cabo en un horno a 40ºC durante 48 horas → ***Capítulo 3.***

Todos los filtros obtenidos se almacenaron en placas Petri de polipropileno previamente enjuagadas con agua destilada filtrada y se dejaron secar durante 48h a temperatura ambiente.

5. Cuantificación y clasificación de las muestras de MPs

Cuantificación directa mediante visualización en un estereomicroscopio Leica 2000, con una magnificación 40X (Masiá et al., 2019). Las partículas identificadas como potenciales MPs se clasificaron de manera dual: en función de su color y forma. Los datos se recogieron en una hoja *Excel* para su posterior análisis estadístico → ***Capítulos 2* y *3.***

6. Identificación química de las partículas cuantificadas

Un porcentaje representativo del total de potenciales microplásticos identificados (en torno al 15-20%) fue analizado por Espectroscopía Infrarroja Transformada de Fourier (FT-iR) en el

Servicio Interdepartamental de Investigación de la Universidad Autónoma de Madrid (Uurasjärvi et al., 2021). El perfil individual de cada partícula fue comparado con la base de datos de referencias de materiales (base de datos de la Universidad Autónoma de Madrid), asignándole un valor de fiabilidad. Ningún resultado con una certeza inferior al 60% fue tenido en cuenta durante el desarrollo de esta Tesis → ***Capítulos 2 y 3.***

7. Evaluación del impacto derivado de la exposición a MPs

Una vez identificados y reconocida la naturaleza química de los materiales analizados mediante el FT-iR, se comprobó su potencial toxicidad (Khosrovyan & Kahru, 2021) para el ser humano y el medio acuático en la página web de la Agencia Química Europea (https://echa.europa.eu/es/home) → ***Capítulos 2 y 3.***

8. Análisis genéticos mediante *barcoding*

Extracción de DNA a partir de Chelex® (Estoup et al., 1996) y amplificación en termociclador mediante PCR-RFLPs. Comprobación del producto de PCR en gel de agarosa y posterior secuenciación (MACROGEN España) → ***Capítulo 3.***

9. Valoración del daño directo sobre la integridad del DNA producido por MPs e influencia en su estado fisiológico

Extracción de DNA genómico del hígado de las merluzas → ***Capítulo 3*** mediante kit de extracción comercial (Blood & Tissue kit, Qiagen, Hilden, Germany). Cuantificación fluorimétrica en Qubit4 y electroforesis en gel de agarosa (1.3%). Tras esto, asignación de valores de degradación según el grado de fragmentación del ADN (Masiá et al., 2021). Finalmente, comparación de estos valores con un estimador del estado fisiológico de las merluzas.

Factor de condición de Fulton (***Capítulo 3***) calculado, según la bibliografía, como

$$k = 100 * \frac{W}{L^3}$$

10. Cálculos estadísticos

Todos los análisis estadísticos fueron realizados con el software libre PAST V.2.17 (Hammer et al., 2001), para un intervalo de confianza del 95% (umbral de significación $p<0.05$), aplicando corrección de Bonferroni en caso de comparaciones múltiples → ***Capítulos 2 y 3.***

Resultados

Capítulo 1:

From the ocean to jellies forth and back? Microplastics along the commercial life cycle of red algae.

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Gelidium corneum. **Fotografía de Luis Fernández García (https://commons.wikimedia.org/wiki/File:Gelidium_corneum_19880601a.jpg)**

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Focus

From the ocean to jellies forth and back? Microplastics along the commercial life cycle of red algae

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Keywords: Microplastics; Red Seaweed; Human health; Industrial activities; Algal production; Gelling industry

Abstract

Red algae are increasingly exploited for direct consumption and for production of gelling agents like agar and carrageenan, widely employed in food and personal care products. In this article we identify knowledge gaps about microplastics in the whole commercial life cycle of gelling red algae, from their marine production to the final wastewater treatment. Recommendations for new research include studies of microplastics deposition on red algae at sea, during the industrial process of production of gelling agents, and indeed about improvements of microplastics retention in wastewater treatment plants.

1. Introduction

Marine litter studies have been on the rise for the last decades, being microplastics (MP) an emerging focus. That is because MPs act like vectors for toxic compounds such as Bisphenol A (Rochester, 2013), an endocrine disruptor, and heavy metals (Brennecke et al., 2016; Bradney et al., 2019; Pachana et al., 2010). These toxics can cause several diseases to marine fauna and finally to the human consumer (Andrady, 2011; Cole et al., 2011; Brennecke et al., 2016). MPs are the most abundant litter particles in the seas (Galgani et al., 2015; Barcelo and Pico, 2020). This is due to the continuous degradation of bigger plastic elements (like fishing nets and strains, see Fig. 1) that form secondary MP (Cole et al., 2011; Carbery et al., 2018a, Carbery et al., 2018b; Setälä et al., 2018), and also to the release of primary MP in cosmetics and industrial products (Lei et al., 2017; Zhou et al., 2020). Primary release MP are incorporated in the product during the industrial process, as in personal care products, detergents, paints, abrasives, agriculture and others (European Commission (DG Environment) intentionally added microplastics in products. https://ec.europa.eu/environment/chemicals/reach/pdf/39168%20Intentionally%20added%20microplastics%20-%20Final%20report%2020171020.pdf). MPs, whose largest length is 5 mm (Arthur et al., 2009), are found floating in the water column (Lavender and Thompson, 2014), deposited on the bottom (e.g. Pabortsava and Lampitt, 2020), and also associated to biota: inside animals (Cole et al., 2014) and attached to the external surfaces of marine plants (Gutow et al., 2016; Goss et al., 2018).

Fig. 1. Macroplastic (Right) and Microplastic (Left) found in El Arañón and Zeluán beaches respectively (Ría de Avilés, Asturias, Northern Spain). Photographs taken by D. Menéndez.

The presence of MP in water and biota leads to their introduction into marine food webs starting in lower levels (Setälä et al., 2018; Goss et al., 2018) and then arriving at higher levels by trophic transfer (Farrell and Nelson, 2013; Carbery et al., 2018a; Nelms et al., 2018; Zhang et al., 2019). Since MPs are not absorbed in the intestinal tract in significant quantities, they do not accumulate in animal organs and tissues (Akoueson et al., 2020); thus the trophic transfer depends on the MPs ingested by the prey at the time of predation (Priscilla et al., 2019), and MP concentration in higher trophic levels may be diluted. For this reason, although significant MPs ingestion has been demonstrated from their presence in faeces (Schwabl et al., 2019), the risk of human consumption of MP via marine animals is debatable, especially from large fish where muscle is the main edible tissue. In contrast, ingestion of primary producers (algae) implies the ingestion of MP deposited on or adhered to their surface, which may pose a risk to the human consumer (Carbery et al., 2018b; Waring et al., 2018). Many studies have been published related to MPs in the marine environment and trophic transfer in animals (e.g. Farrell and Nelson, 2013; Chagnon et al., 2018). However, some marine species that are highly consumed by humans have not yet received much attention regarding the risk of MP transfer to humans: red algae.

2. Red algae and their gelling agents

Red algae (Rhodophyta Wettstein, 1901) is an algal division mainly comprised by macroalgae (Díaz-González et al., 2004). They are highly consumed worldwide due to their nutritional benefits (Kumar et al., 2008). According to Díaz-González et al. (2004), the genus *Porphyra* is the most important because its species are widely cultivated for food production: Nori in Japan, Purple Laver in Wales. *Palmaria palmata* is also intensely cultivated for its antioxidant benefits (Yuan et al., 2005b; Yuan et al., 2005a).

Other red algae of high interest for humans are gelling agents' producers: agarophytes and carrageenaphytes (http://www.fao.org/3/y4765e/y4765e04.htm#bm04.3). Agar and Carrageenan, respectively, are the gelling agents extracted from them (Usov, 1998). These compounds are sulphated polysaccharides present in the cell wall, where they represent 15%–30% of dry weight in Agar producers and 30%–80% in carrageenan producers (Rioux and Turgeon, 2015). Agar is principally extracted from the following species: *Gelidium sesquipedale*, *Gracilaria* (*G. gracilis*, *G. dura* and *G. bursa-pastoris*), *Gelidiella acerosa*, *Pterocladia capillacea* and *Anhfeltia plicata* (Salt, 1976; Prasad et al., 2007; Lee et al., 2017; Titlyanov et al., 2017; Lebbar et al., 2018) and carrageen from *Chondrus crispus*, *Mastocarpus stellatus*, *Kappaphycus alvarezii*, *Gigartina* (*G. decipiens*, *G. skottsbergii* and *G. stellate)*, *Sarcothalia crispate* and *Eucheuma striatum* (Craigie, 1978; Epifanio et al., 1981; Díaz-González et al., 2004; Tasende et al., 2013; Hughes et al., 2018).

Gelling agents are commonly used in research (functional biology, pharmaceutics, molecular biology…) (Bhattacharya et al., 1994; Shilpa et al., 2011) and in cosmetics and food industry (Aliste et al., 2000; Joshi et al., 2018). Moreover, algal gelling agents are a perfect substitute for animal gelatine. Both Agar (E-406) and Carrageenan (E-407) are commonly used as food additives due to their gelling, thickening and nutritional properties (Rioux and Turgeon, 2015; Cunha and Grenha, 2016; Lee et al., 2017). Seaweed species are harvested to elaborate vegan, vegetarian and religion-friendly products (e.g. halal, kosher), and to reduce animal exploitation. The industry of gelling red algae products is estimated to produce more than 370 million dollars in benefits according to FAO (http://www.fao.org/3/y5600s/y5600s07.htm). Due to their wide use, it is important to know their role in the transfer of hazardous elements like MP from the ocean to consumers.

3. MP and red algae, what do we know?

Fig. 2 represents the cycle of red algae where MP can be transported. The cycle starts with the adhesion of MP to the algal surface (step 1), which, although not much studied yet, has been demonstrated *in Fucus vesiculosus* (Gutow et al., 2016), *Pyropia* sp. (Li et al., 2020), *Endocladia muricata* (Saley et al., 2019) and others. Then the algae production (step 2) will introduce more MP because nets, boxes and other tools employed to cultivate or harvest red algae are made of different types of plastic (Li et al., 2016; Simeonova and Chuturkova, 2020). Red algae can be directly consumed – sold fresh or dry for human consumption, so humans will directly ingest MP carried by them. Alternatively, they can be industrially processed (step 3) to extract the gelling compounds and use them in different fields (cosmetics, food). Those compounds will contain the MP carried by red algae, and others may be added purposely (primary MP), for example in cosmetics like tooth paste and scrubs (Napper et al., 2015; Duis and Coors, 2016; Anagnosti et al., 2021). Finally, either consumed directly or as part of gelling industry, MP contained in red algae will be part of wastes/debris present in wastewater to be treated in Wastewater Treatment Plants (WWTP). Hospido et al. (2004) call WWTP "ecological treatment systems" where pollutants are removed from water before it is returned to the environment. Although their efficiency of MP retention can be up to 90% or even higher, a part of them escapes their retention systems (Murphy et al., 2016; Masiá et al., 2020), and will return to the sea (step 4).

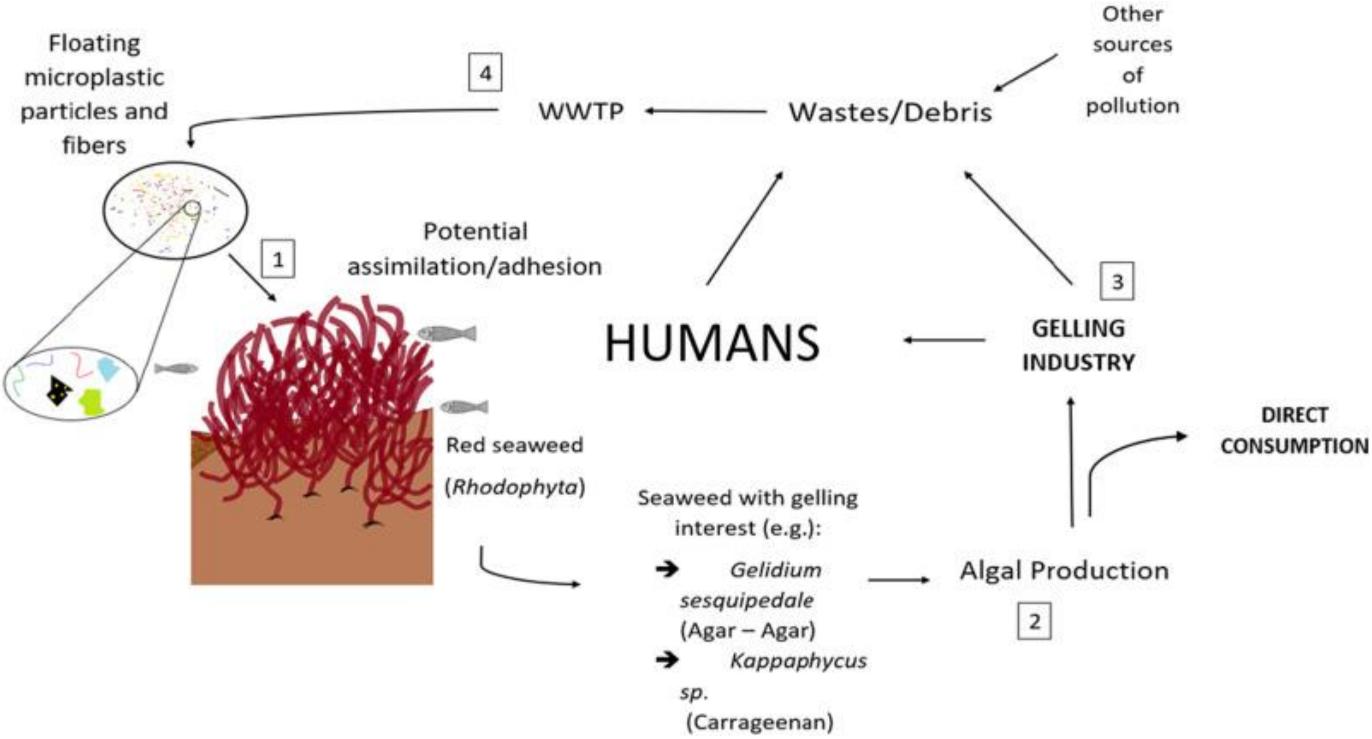


Fig. 2. The MPs cycle through red algae used by humans. Steps 1, 2, 3 and 4 are critical for MP increase or retention.

Here we consider only the regular, legal cycle; in addition, we could add those industrial and household wastes that are irregularly treated, directly dumped, or accidentally deposited in rivers and seas. These have been estimated to be the majority of wastewater volumes in some regions (https://www.unwater.org/water-facts/quality-and-wastewater-2/).

4. MP and red algae, what do not we know?

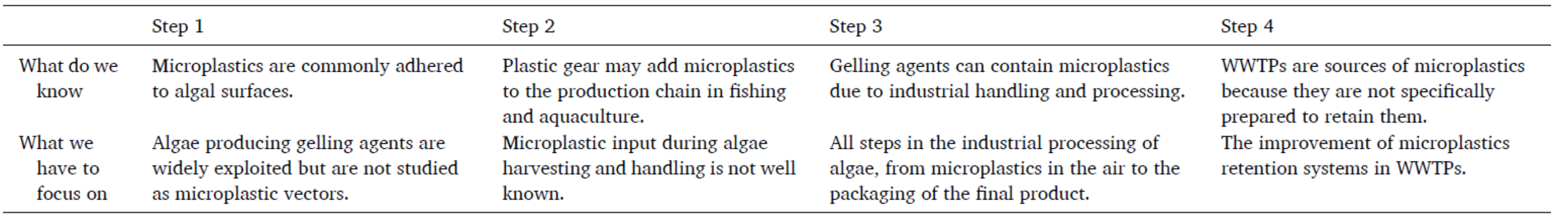
The first gap of knowledge in step 1 (Fig. 2) is the lack of information of MP content in many species of red algae exploited for direct human consumption and for gelling compounds. Due to their use in food and cosmetic industry (Duis and Coors, 2016; Welden and Cowie, 2017) their study from the point of view of MP contents is considered essential (Guerranti et al., 2019). However, according to Li et al. (2020), very few studies have been published to evaluate how MPs affect macroalgae and how those MPs are transferred to the food web. Studies reporting MP on red algae at sea are limited to a few edible species such as *Porphyra* sp. (Li et al., 2020). Many more studies are needed about MP content in red algae, especially in important genera such as *Gelidium*, *Gracilaria*, *Kappaphycus*, *Euchema* and others.

The second knowledge gap occurs in Step 2 (Fig. 2). The MPs produced during the extraction of red algae (and in general of seaweed) are still not well known. Plastic nets and protection gloves (made of neoprene) are commonly used in fishing and aquaculture industry to manipulate all products, and are considered sources of MP (Montarsolo et al., 2018). Elements used in red algae harvesting and handling processes could be considered MP pollution sources, but currently there is another lack of information here (Duis and Coors, 2016).

In step 3 (Fig. 2) there is another gap. Although gelling products are widely used, MPs non-intentionally introduced during their industrial life cycle have not been considered yet. For example, air floating MP are a pollution source in all environments (Gasperi et al., 2015; Gasperi et al., 2018), and is logically expected to occur in the industry of gelling products. It is difficult in the industrial environment to avoid this pollution due to the current great dependence on plastic. Also, post-industrial processes such as packaging in plastic bags can be considered as a MP source (Wagner, 2017; Gerritse et al., 2020). Moreover, although products with primary MP have been thoroughly studied (e.g. Leslie, 2014; Duis and Coors, 2016; Guerranti et al., 2019), Agar and Carrageenan have yet to be examined as potential sources of MP in human diet to date.

Knowledge gaps about MPs, summarized in Table 1, occur at the WWTP level too (step 4 in Fig. 2). Indeed, these gaps are not specific of MP derived from red algae but general and can be applied to all MPs. As explained above and in other studies (Ziajahromi et al., 2016; Edo et al., 2020), the improvement of WWTP systems for MP retention is needed to ensure they do not enter into running waters and arrive in the sea.

Table 1. Summary of needs and gaps in the cycle of gelling agents' producers. WWTPs: wastewater treatment plants.



	Step 1	Step 2	Step 3	Step 4
What do we know	Microplastics are commonly adhered to algal surfaces.	Plastic gear may add microplastics to the production chain in fishing and aquaculture.	Gelling agents can contain microplastics due to industrial handling and processing.	WWTPs are sources of microplastics because they are not specifically prepared to retain them.
What we have to focus on	Algae producing gelling agents are widely exploited but are not studied as microplastic vectors.	Microplastic input during algae harvesting and handling is not well known.	All steps in the industrial processing of algae, from microplastics in the air to the packaging of the final product.	The improvement of microplastics retention systems in WWTPs.

5. Conclusions and recommendations

Red algae production is in great demand because their derived products are widely employed in daily life. Here we have detected knowledge gaps in several steps of the life cycle of commercial red algae regarding their content of MP, from primary production to the treatment of final wastes. Recommendations for future studies begin with the analysis of MP deposition on or adherence to all exploited species of red algae. Subsequent studies could address their industrial handling during harvesting and aquaculture, as well as the sources of contamination in the process of obtaining agar and carrageenan. The amount of MP in agar and carrageenan should be analysed given their wide use in food products. Finally, WWTPs should continue to be studied in order to improve their efficiency of MP retention systems.

CRediT authorship contribution statement

Conceptualization, Daniel Menendez, Alba Ardura, Eva Garcia-Vazquez; Bibliographic search, Daniel Menendez and Eva Garcia-Vazquez; Writing – first draft, Daniel Menendez; first review, Alba Ardura and Eva Garcia-Vazquez; review & editing, all authors.

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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Capítulo 2:

Microplastics across biomes in diadromous species. Insights from the critically endangered *Anguilla anguilla*

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***Anguilla anguilla*. Fotografía de D. Menéndez.**

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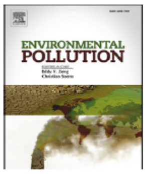

Microplastics across biomes in diadromous species. Insights from the critically endangered *Anguilla anguilla*☆

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Keywords: Diadromous species; European Eel; Harmful chemicals; Marine Pollution; Microplastics; Riverine pollution

Abstract

Microplastic pollution affects freshwater and marine biota worldwide, microplastics occurring even inside the organisms. With highly variable effects, from physical damage to toxicity of plastic compounds, microplastics are a potential threat to the biodiversity, community composition and organisms' health. This emerging pollutant could overstress diadromous species, which are exposed to both sea and river water in their life cycle. Here we have quantified microplastics in young European eel *Anguilla anguilla*, a critically endangered catadromous fish, entering three rivers in southwestern Bay of Biscay. River water, sediments and seawater were also analysed for microplastics. The microplastic type was identified using Fournier-Transform Infrared spectroscopy and then searched for their hazard potential at the European Chemical Agency site. Both riverine and sea microplastic pollution were predictors of eels’ microplastic profile (types of microplastics by shape and colour): *A. anguilla* juveniles entering European rivers already carry some marine microplastics and acquire more from river water. Potentially hazardous plastic materials were found from eels, some of them dangerous for aquatic life following the European Chemical Agency. This confirms microplastics as a potential threat for the species. Between-rivers differences for microplastics profiles persistent over years highlight the convenience of analysing and preventing microplastics at a local spatial scale, to save diadromous species from this stressor. Since the origin of microplastics present in glass eels seems to be dual (continental + seawater), new policies should be promoted to limit the entry of microplastics in sea and river waters.

1. Introduction

Plastic debris is globally recognized as a pervasive environmental issue, being increasingly found in nearly every habitat, from mountains (Allen et al., 2019) to rivers and oceans (Depledge, 2013; Yonkos et al., 2014), posing a threat to most living organisms (Derraik, 2002; Martins & Sobral, 2011; Hammer et al., 2012; Wagner et al., 2014; Li et al., 2020a, 2020b; Sarijan et al., 2021). Mechanical and physical-chemical degradation processes break plastic litter into smaller pieces (Thompson et al., 2004), called microplastics (MPs thereafter) when their size is < 5 mm (Gago et al., 2015); besides, many plastic items (primary MPs) are produced of this size for use in cosmetics and cleaning products (Cole et al., 2011; Andrady, 2017). MPs are ubiquitous pollutants in all marine and freshwater ecosystems across the globe (Rochman, 2018). For their small size MPs are easily transported and many of them cannot be retained in wastewater treatment plants (WWTP) (Murphy et al., 2016; Masiá et al., 2020). Rivers, fishing activities, wastewater discharges of large urban concentrations, are important contributors to marine MPs (e.g., Browne et al., 2011; Lebreton et al., 2017; Masiá et al., 2021; Meijer et al., 2021).

Given MPs occurrence in both freshwater and marine environments, the species that occupy both biomes (diadromous species like salmon or eel) are exposed to microplastics from both environments, particularly when they are in the interface of marine and continental waters. Diadromous fishes undertake long migrations and experience changes to adapt to different salinities, being especially vulnerable to environmental disturbances due to their dual habitat (Tamario et al., 2019). In addition to drastic extinctions glass eels have undergone in the past century (e.g., Merg et al., 2020), they are currently threatened by global change (Nyboer et al., 2021). Anthropogenic disturbances in rivers are of main concern, and management measures for conservation of diadromous fish are targeting principally the freshwater stage of their life cycle (Verhelst et al., 2021).

The European eel *Anguilla anguilla* L. is a catadromous species (reproducing at Sargasso Sea and growing to maturity in a river) critically endangered according to the International Union for Nature Conservation (see the species' page in the European Commission website https://ec.europa.eu/oceans-and-fisheries/ocean/marine-biodiversity/eel_en, accessed on April 2022). European eel populations are drastically reduced in the Atlantic (Dekker et al., 2007), Baltic (Andersson et al., 2012) and Mediterranean (Aalto et al., 2016) basins. Being a catadromous species that reproduces in the ocean and grows in the river, it is affected by changes in the two habitats. Its recruitment has declined due to reduced spawning stock (Dekker, 2003), caused by climatic and hydrology changes, overfishing, parasitic infections, loss of habitat due to barriers in the rivers, among others (e.g., Feunteun, 2002; Starkie, 2003; Wirth and Bernatchez, 2003; Kettle et al., 2011; Meulenbroek et al., 2020). Environmental pollution is also contributing to this severe decline in synergy with the rest of factors (e.g., Maes et al., 2005; Geeraerts et al., 2011; Jacoby et al., 2015; Drouineau et al., 2018).

Regarding the importance of pollution in the decline of European eel, the emerging MPs are surely an additional stressor for this species. European eel is likely to be exposed to MPs during its life cycle. However, only Steer et al. (2017) reported MPs in *A. anguilla* larvae in the western English Channel. This is a serious call for attention in a critically endangered species. MPs have adverse effects on fish health, varying from deterioration of the nutritional status for the feeling of satiation (e.g., Wright et al., 2013) to toxic effects that affect different organs and hamper metabolic and neurological functions and fish development (e.g., Wang et al., 2020), alone or together with other adsorbed pollutants (e.g., Barboza et al., 2018; Gola et al., 2021). The fish health risks associated MP exposure, the scarcity of studies of MPs in *A. anguilla* and its critically endangered status of conservation, make it urgent to expand the research of MPs in this species that is a good example of vulnerable diadromous fish.

Here we have quantified and analysed the composition of MPs in glass eels, i.e., eel juveniles arriving in river mouths of south Bay of Biscay after their travel from the nursery in Sargasso Sea (van Ginneken & Maes, 2005). In that moment they are exposed to both marine and riverine microplastics. Our hypotheses were:

a) Glass eels will contain MPs acquired at sea, because MPs have been widely found in other fish inhabiting in the North Atlantic Ocean (e.g., Barboza et al., 2018);

b) MPs will be also acquired from the rivers glass eels are entering, rivers being recognized sources of MPs pollution (e.g., Kumar et al., 2021);

c) The composition of glass eels MPs will be similar to that of the biome (sea or continental waters) contributing the most to young eel MPs content.

2. Materials and methods

2.1. Ethics statement

The eels analysed here, kindly provided by fishing guilds, had been caught from artisanal fisheries for further commercialization. Therefore, eel samples were not detracted from the wild for this study and were already dead. Being commercial seafood, specific permit for sampling was not necessary. The project about microplastics has been approved by the competent Research Ethics Committee of Asturias Principality with the reference CEImPA 2021.116.

2.2. Species biology

A. anguilla is a catadromous fish with its spawning zone in the Sargasso Sea, in the central-western Atlantic Ocean. Larvae travel to European rivers to grow in freshwaters. Various larval stages occur during the trans-oceanic journey that can last from 1 up to 2.5 years, entering the rivers when they acquire the glass eel stage (van Ginneken & Maes, 2005), though some authors have described juveniles leptocephali eel larvae to last up to 280 days for this migration (Lecomte-Finiger, 1994). Once in the rivers, the maturation time of the individuals varies depending on the sex. Males may spend between 6 and 12 years and females between 9 and 20 years in yellow eel stage in freshwater, leaving the rivers when they are completely mature to travel back to the Sargasso sea to reproduce (Deelder, 1970). After the reproduction, the eels die.

2.3. Samples and sampling sites

Despite its endangered conservation status, European eel is legally caught and commercialized in European countries. In Asturias (southwest Bay of Biscay, Spain), fishing guilds gave samples of glass eels caught legally (https://www.mapa.gob.es/es/pesca/temas/planes-de-gestion-y-recuperacion-de-especies/plan%20de%20gesti%C3%B3n%20anguila_Asturias_tcm30-282056.pdf) from the mouth of three rivers: Nalón, Bedón and Cabra rivers (marked with stars in Fig. 1), in the season of commercial eel fishing (late autumn-winter of 2020). Nalón River basin is one of the largest in Asturias with 141 km, 3693 km^2 of basin surface and an average flow of 55.18 m^3/s. The river crosses forests and agriculture fields upstream, the main coalmining zone of Asturias in its mid reach and has industry and two fishing ports in the lower reach. A WWTP is located 20 km upstream the river mouth. The Bedón is a small coastal river of 19.1 km, a basin of 126.6 km^2 and average flow of 3.04 m^3/s flowing through an area with agriculture. No WWTP nor fishing ports are near the river mouth. The Cabra, also in a rural zone with agriculture, is the smallest of these three rivers being 5 km long with a basin of 28.1 km^2 and very irregular flow. Near its mouth there is a WWTP, about 200 m upstream the sampling point; no fishing port near the mouth. More information about the hydrology of Asturias rivers can be found on the web of the Hydrographical Confederation of the Cantabrian Sea (https://www.chcantabrico.es/organismo/las-cuencas-cantabricas/marco-fisico/hidrologia). The location of WWTPs in Asturias can be found on https://consorcioaa.com/saneamiento/.

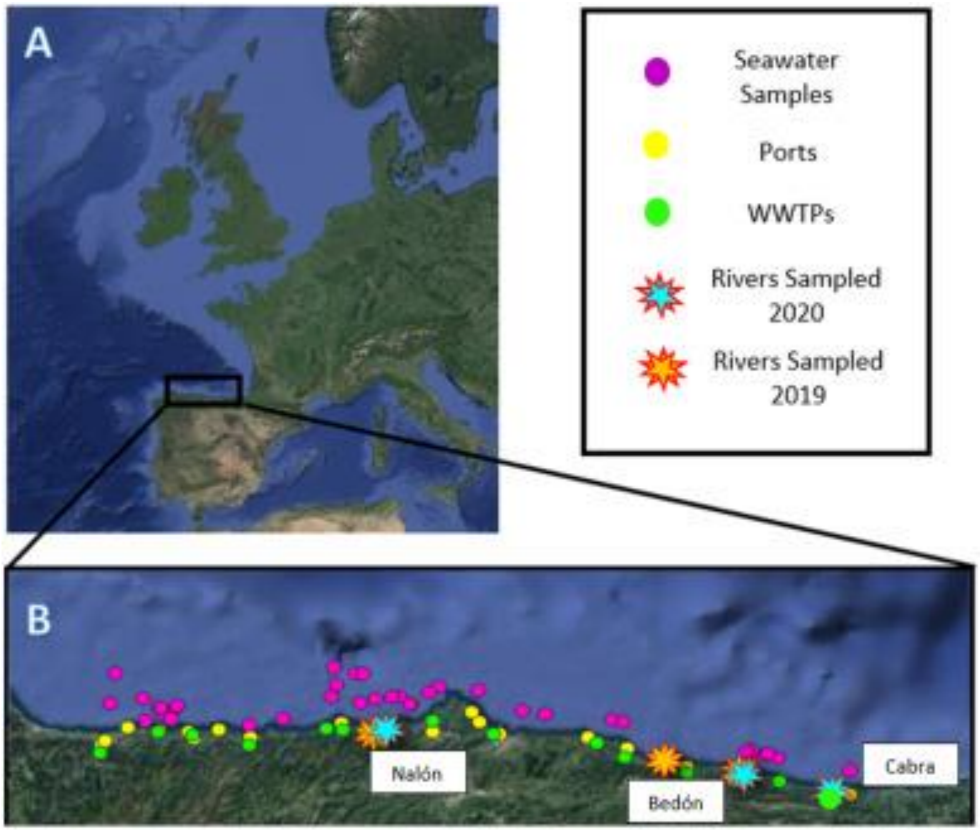

Fig. 1. Sampling area in Asturias coast. A) European position of the sampling area. B) Rivers with eels, water and substrate sampled in 2020: blue stars (from left to right: Nalón, Bedón and Cabra rivers). Seawater samples: pink dots. River substrate sampled in 2019: orange stars (from left to right: Aguilar, Vega and Gulpiyuri). Wastewater treatment plants (WWTPs): green dots. Fishing ports: yellow dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Water samples were obtained from the rivers and from the sea. Surface water samples of each river were collected in four 5 L bottles (20 L per river), previously cleaned with filtered water, at three points close to the river mouth where glass eels were caught (blue stars in Fig. 1). Seawater samples (N = 48) were taken by fishermen from fishing boats in 5 L bottles (240 L in total) rinsed with filtered water, at different distances from the coast (pink dots in Fig. 1, some of them overlapped).

Samples of substrate (sand) were taken from the mouth of the three rivers adapting the protocol described by Besley et al. (2017). Briefly, 20 glass jars were fulfilled (150–300 gr of wet sand) in random points along a 100 m transect parallel to the riverside as close as possible to the river mouth. Samples were stored in the fridge until processing.

Fifty glass eels of each river sample were taken at random, weighted and measured, and its pigmentation stage determined from visual examination following Elie et al. (1982) and Briand

(2009). The rest of samples (55 individuals-19.9 g, 110 individuals-40.2 g and 65 individuals-22.7 g of glass eels for Nalón, Bedón and Cabra rivers respectively) were rapidly stored in glass bottles at −20 °C, avoiding to expose them to aerial MPs (Gasperi et al., 2018).

2.4. Microplastics extraction

Water samples were filtered immediately when arrived at the laboratory through 0.45 µm pore size PES (polyethersulphone) filter (PALL Corporation).

To extract MPs from the substrate, 50 g of dried substrate per sample (1000 g per river mouth) were added to a 200 mL hypersaline solution (358.9 g of NaCl/L of filtered water) (Besley et al., 2017). The mixture of substrate and hypersaline solution was manually shaken for 2 min, then let to rest for 24 h (Laglbauer et al., 2014). The liquid solution containing the MPs was carefully filtered with a 0.45 µm pore PES filter avoiding sand to fall (Masiá et al., 2021). Despite of its limitations - it excludes separation of the densest particles such as PVC - it is presented as a method easy to perform requiring low amount of sophisticated equipment, enabling it to be easily used, and making it feasible for less developed countries and, even citizen science projects; which facilitates its use as a standardized method (Besley et al., 2017).

Complete glass eels were first digested following a modification from the protocol described by Li et al. (2020a). Briefly, 20 mL of H_2O_2 were added per gram of eel tissue for digestion in glass jars. Jars were covered with aluminium foil and placed in a static oven at 60 °C for 7 days to be sure that all organic material was dissolved. Manual agitations were carried out daily to move the residue anchored to the walls of the glass jars. Once the digestion process was completed, the solution was diluted until 1 L with filtered water to prevent filters to clog. Five PES filters (0.45 µm pore) were finally obtained per sample (200 mL per filter).

All filters were immediately stored in petri dishes, properly covered, and dried at room temperature in closed chambers for 48 h before counting MPs.

Measures to ensure contamination control were taken. Researchers wore cotton overalls. In the laboratory, all the materials (tweezers, vacuum pump, glass jars, petri dishes and plates) were previously cleaned with filtered distilled water (0.2 µm pore size PES filters) before every contact with each specimen (Lusher et al., 2015). The digestion reagent, H_2O_2, was filtered too before use (0.2 µm pore size PES filter). Blanks prepared without eel tissue – only with filtered reagents and distilled water-were run simultaneously with the samples to control procedural MPs contamination. All the procedures were carried out inside a closed laminar flow chamber to prevent airborne MPs contamination.

2.5. Microplastics identification and composition analysis

Filters were directly observed under a stereomicroscope at 40x magnification following Masiá et al. (2019). Plastic particles recognized were not longer than 5 mm length (Andrady, 2011). The first classification was carried out to differentiate between plastic fibers (elongated particles, normally cylindrical), plastic fragments (irregular shape particles, sometimes multi-coloured) and microbeads (spherical particles) following Hidalgo-Ruz et al. (2012) and other authors (Cole, 2016; Tanaka & Takada, 2016; Miraj et al., 2019) (Fig. 2). Then, plastics were counted and included in groups according to their colour. As performed in many studies, particles coloration comparison was carried out to evaluate the different pollution between samples (e.g., Kovač-Viršek et al., 2016).

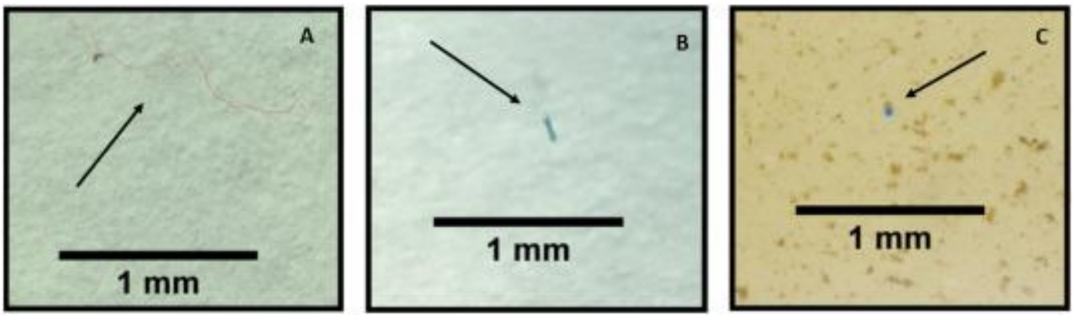

Fig. 2. Examples of the types of microplastics found in this study. A, Red fiber. B, Blue fragment. C, Blue microbead. Photographs taken by D. Menéndez. Scale: 1 mm (ImageJ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

To put the results in context and check if the MPs spatial pattern is maintained over time, we compared the results obtained in this study with previous data, using the MPs profiles of the beaches closest to each river as a proxy – in absence of data from exactly the same locations. This was done with substrate data obtained in the winter of 2019, published in Masiá et al. (2021). Sampling was done during the cold season in the two studies, when beach cleaning is not done. Amongst the beaches analysed in Masiá et al. (2021), Aguilar, Vega and Gulpiyuri are the closest to Nalón, Bedón and Cabra rivers, thus their data were compared with those of river substrate of this study.

Around 17% of the items visually identified as putative MPs were analysed for chemical composition as recommended by Uurasjärvi et al. (2021). At least one random representative from each group (fibre, particle and microbead; Fig. 2), colour and element (water, substrate, eels) were included. Fournier-Transform Infrared spectroscopy (FTIR) was employed using FTIR Varian 620-IR and Varian 670-IR, Germanium Glass, 4000–500 cm^{-1}. Apparently there was no organic matter remaining on the MP surface because no interference was detected in the FTIR analysis.

Health risks of the materials found were checked in the European Chemical Agency (ECHA) webpage at https://echa.europa.eu/home.

2.6. Statistical analysis

MP data were transformed to MP/g in all the elements (water, substrate, eels) to make the results comparable. Due to the small sample size in some MPs categories and limited number of samples analysed, nonparametric tests were preferred for comparisons between samples. Chi square test was used to compare differences among groups of samples for the types and composition of the particles (putative MPs) found. Kruskal-Wallis test was employed to compare the concentration of MPs between the elements (water, substrate, glass eels) analysed in the three rivers. Normality was checked using Shapiro-Wilk test, and homogeneity of variance using Breusch–Pagan' test.

Multiple regression analysis was carried out to determine the factors (profile of MPs in each environmental component –river water, river substrate, seawater-as independent variables) that explain significantly the profile of MPs in glass eels (dependent variable).

Spearman's rank correlation was used to test if the spatial pattern – the relative abundance of each MP type in the three zones considered-was similar in consecutive years. For this, rivers were ranked for each MPs type (distributing the MPs in blue fibres, black fibres, white fibres, and other particles, to harmonise the results with those of Masiá et al., 2021) in 2019 and 2021, and the correlation between years was calculated together with its significance.

Standard threshold of $p < 0.05$ for significance was employed. Statistics was performed using PAST free software v.2.17 (Hammer et al., 2001).

3. Results

3.1. Data overview

In total 105 eels from Nalón River, 160 from Bedón River and 115 from Cabra river were obtained. The 50 glass eels analysed morphologically from each river provided similar results per river regarding weight and length. For the pigmentation stage, most glass eels were in stage VB, with more eels in further stages in Cabra River (Supplementary table 1).

A total of 55 eels from Nalón River (19.9 g as stated above), 110 from Bedón River (40.2 g) and 65 eels from Cabra River (22.7 g) were analysed for MPs. Abundant putative MPs (n = 926 items including the two visible items described above) were found in this study, considering all types of samples and locations (Supplementary Table 2). The lowest concentration in MPs per gram corresponded to seawater (0.0002 MP/g) and the highest to glass eels of Cabra River (2.74 MP/g)

(Table 1). In the three rivers, MP concentration increased from water to glass eels (water < sediments > glass eels). All elements were significantly different (Kruskal-Wallis test with tie corrected $Hc = 7.2$, $p = 0.027 < 0.05$).

Table 1. Concentration of MPs (MPs/g) in water, sediment and glass eels in the locations examined. Standard deviation in parentheses.

Empty Cell	**Water**	**Sediment**	**Glass eels**
Nalón	**0.003 (0.002)**	**0.035 (0.017)**	**2.665 (0.203)**
Bedón	**0.005 (0.003)**	**0.059 (0.015)**	**2.54 (0.053)**
Cabra	**0.018 (0.019)**	**0.051 (0.016)**	**2.736 (0.061)**
Marine locations	0.0002 (0.0001)	–	–

3.2. Inferred acquisition of MPs by glass eels

Two individuals from Cabra River contained MPs in the dorsal muscle under the skin (Fig. 3), in a place that these items can reach only during earlier stages of development at sea (muscle development). Those MPs were visible by the naked eye without the need of magnifying glasses or microscope and were manually recovered using a superficial cut to not sever the digestive tract. After removing these two visible MPs, the two eels were processed together with the rest of Cabra River eels. The rest of eels from the same and the other two rivers did not exhibit MPs visible through the skin without augmenting lenses.

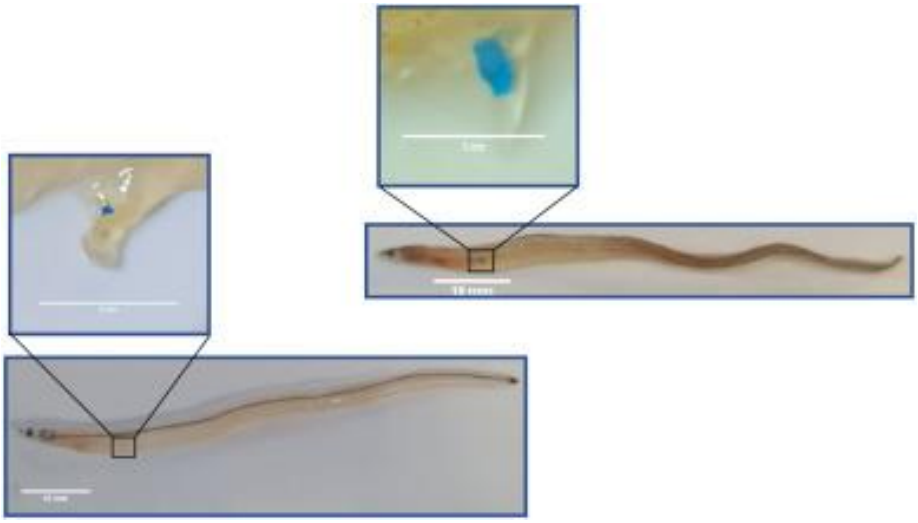

Fig. 3. Eels from Cabra River with visible the superficial dissection showing two blue particles of urethane alkyd. Photographs by D. Menéndez.

The type (shape and colour) of MPs served to infer the main origin of MPs acquired by glass eels. Most putative MPs found in the rivers studied here were blue and black fibres, while fragments were clearly less abundant (Fig. 4). The proportions of the different types of MPs by shape and colour were significantly different between rivers: in sediments ($\chi^2 = 21.3$, 12 degrees of freedom (d.f.), $p = 0.04$), water ($\chi^2 = 67.9$, 16 d. f., $p = 0.0002$) and glass eels ($\chi^2 = 25.3$, 14 d. f., $p = 0.03$). MPs profile in seawater was different from those of both glass eels and river environment (Fig. 4), with a higher proportion of fragments and less blue and black fibres in seawater in comparison with rivers ($\chi^2 = 49.3$, 58.1 and 163, all with 6 d. f. and $p < 0.001$, for the comparisons of seawater versus Nalon, Bedon and Cabra river water respectively).

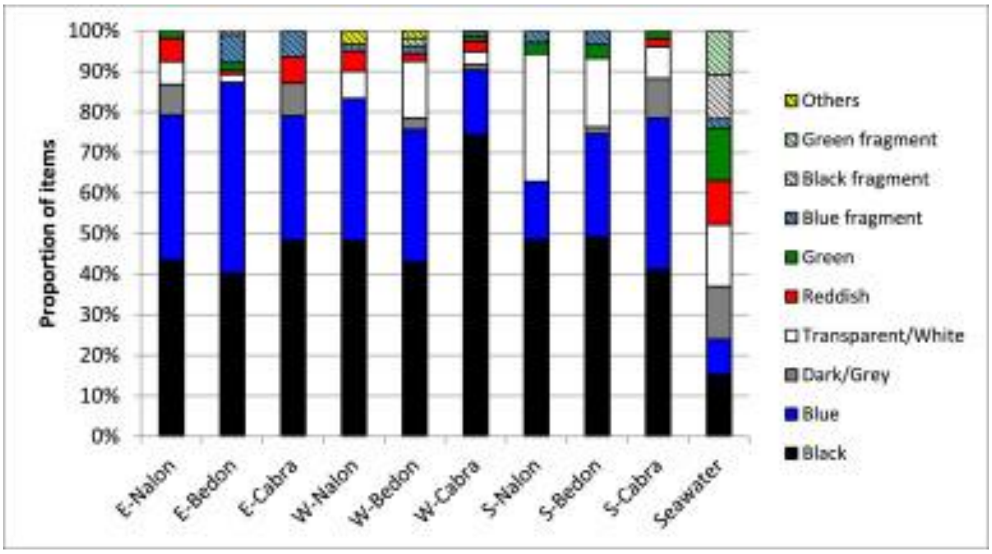

Fig. 4. Profile of MPs by shape and colour in samples of glass eels (E), water (W) and sediment (S) from Nalón, Bedón and Cabra rivers, and in the seawater samples analysed. Fragments are marked with stripes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Multiple regression analysis with the MPs profile in eels as dependent and in environmental components as independent variables showed that the river water profile explained significantly the glass eels profile in the three rivers (Supplementary Table 3). Sediment profile was also significantly correlated with that of glass eels after controlling the rest of variables in Cabra River, and the same happened with seawater in Nalón River, suggesting that glass eels may take the MPs not only from river water.

In order to understand if the apparent spatial distribution between rivers was constant over time, obtained results were compared with those obtained in 2019 from nearby beaches. Although different in the two years, the profiles were generally consistent (Fig. 5). Nalón River zone had a higher proportion of white and transparent fibres while a lower proportion of blue ones than the

Bedón River zone, and Bedón River zone had the same compared to Cabra River zone. Cabra River zone sediments exhibited more fragments than the other two in both 2019 and 2021. The correlation for the relative proportion of these four MP types between 2019 and 2021 was highly significant (r = 0.86, 10 d. f., *p* = 0.0004). Indeed, the glass eels exhibited similar patterns, although the proportion of blue fibres in eels from Nalón and Bedón rivers was higher than in those of Cabra River (blue fibres were more abundant in Cabra River water in both 2019 and 2021).

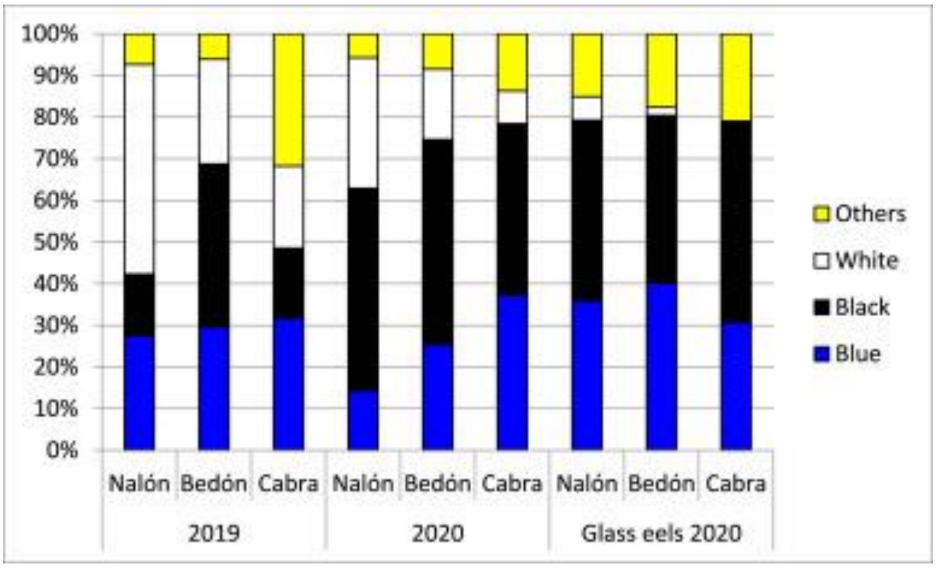

Fig.5. MPs profile of sediments in the zone of Nalón, Bedón and Cabra rivers in 2019 (taken from Masiá et al., 2021) and 2020, and in glass eels sampled in 2020.

3.3. MPs composition in this study and their potential risks

The 17.4% of the particles counted were analysed using FTIR (161 items), from which 130 (80.7%) were of artificial material and the rest were principally alpha-cellulose, and other natural materials. Of those artificial particles 62 were found from water, 34 from sediments and 34 from eels.

The FTIR analysis showed that the two particles (1.5% of the artificial particles) found in dorsal muscle of two Cabra River eels were of urethane alkyd, a type of polyester resin that is used in boats and ships paints. The rest of artificial items (Supplementary table 4) were rayon (artificially transformed cellulose, 46.9%), polyethyleneimine - PEI (17.7%), polyethylene terephthalate - PET (6.2%), polyester (6.9%), polypropylene - PP (3.8%), acrylic/poly-acrylic -PAN/PAA (5.4%), polyethylene - PE (7.7%), and polyethylene glycol - PEG (3.8%). PEG may be taken with caution because PEGs of low molecular weight are soluble in water at 20 °C (https://onlinelibrary.wiley.com/doi/pdf/10.1002/3527600418.mb2532268kske0010, accessed on April 2022), a temperature that can be reached in water in the studied coast in summer.

Chemical MPs profiles were significantly different in the samples analysed ($\chi^2 = 104.03$, 63 d. f., $p = 0.0009$). The items of these materials were unequally distributed in seawater, river water, river sand, and glass eels (Fig. 6). The profiles of glass eels were apparently more similar to those of the same river water than to the sediments and the seawater profiles.

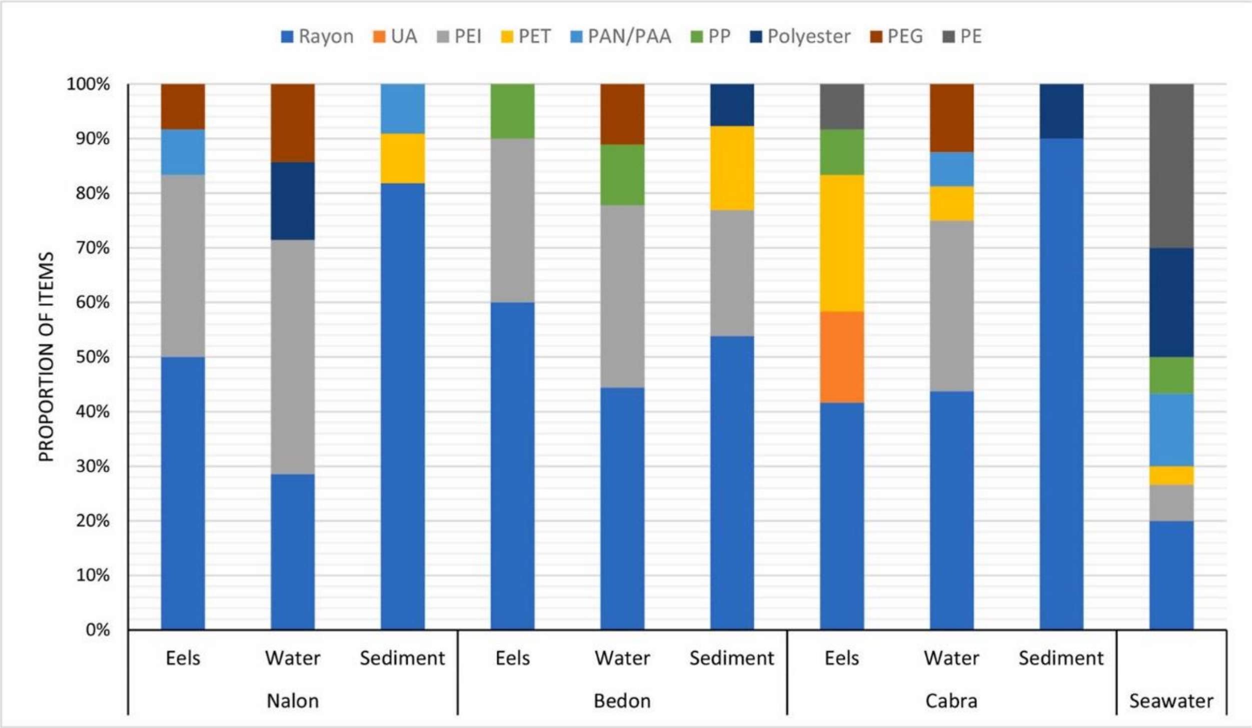

Fig. 6. Proportion of items of different materials found in the samples analysed in is study. UA, urethane alkyd; PEI, Polyethyleneimine; PE, polyethylene; PEG, polyethylene glycol; PAN/PAA, acrylic or poly-acrylic; PET, polyethylene terephthalate; PP, polypropylene.

Multiple regression with glass eels profiles as dependent variables and environmental profiles as independent variables confirmed significant variation of glass eels depending on river water MPs materials for Nalón and Bedón rivers but not for Cabra River (Table 2), where the two urethane alkyd items we likely introduced in dorsal muscle of eels during the development in distant ocean waters. In addition, Nalón River sediments were also significantly explained from sediment MPs composition, suggesting again that many glass eels MPs come from the river.

Table 2. Multiple regression analysis with the profiles (by type of chemical material) of MPs in glass eels and in environmental components as dependent and independent variables respectively. Significant values are marked in bold.

Dependent variable: Nalón eel profiles	**Coefficient**	**SE**	**t**	**p-value**	**R^2**
Constant	**−0.06**	**0.48**	**0.12**	**0.91**	
Nalón water	**1.23**	**0.26**	**4.76**	**0.009**	**0.67**
Nalón sediments	**0.42**	**0.1**	**4.39**	**0.012**	**0.61**
Seawater	**−0.03**	**0.09**	**0.29**	**0.79**	**0.004**
Dependent variable: Bedón eel profiles					
Constant	**−0.66**	**0.26**	**2.49**	**0.07**	
Bedón water	**0.95**	**0.19**	**5.07**	**0.007**	**0.92**
Bedón sediments	**0.3**	**0.12**	**2.52**	**0.07**	**0.86**
Seawater	**0.09**	**0.05**	**1.75**	**0.16**	**0.02**
Dependent variable: Cabra eel profiles					
Constant	**1.33**	**1.08**	**1.23**	**0.28**	
Cabra water	**−0.20**	**0.33**	**0.59**	**0.58**	**0.29**
Cabra sediments	**0.63**	**0.29**	**2.15**	**0.09**	**0.65**
Seawater	**−0.13**	**0.20**	**0.63**	**0.57**	**0.03**

For the potential risk of MPs for glass eels in the zone studied, four of the chemicals identified from FTIR are catalogued as harmful by the ECHA: PEI, PEG, acrylic (PAN/PAA) and polyester (Table 3 - marked in bold). Polyester was not found in MPs analysed from glass eels, but PEI, PEG and acrylic were found in eels, PEI –catalogued as harmful for aquatic life-being the most abundant (Fig. 6, Supplementary table 4).

Table 3. Substances identified in the MPs analysed and their risks, by ecosystem component examined. UA, urethane alkyd; PEI, Polyethyleneimine; PE, polyethylene; PEG, polyethylene glycol; PAN/PAA, acrylic or poly-acrylic; PET, polyethylene terephthalate; PP, polypropylene.

Substance	Risks from ECHA	Eels	Water	Sediment	Seawater
PP	No hazard reported	X	X		X
PET	Under evaluation (pre-registered)	X	X	X	X
PE	No hazard reported	X			X
PEG	Irritative	X	X		
PAN/PAA	Irritative, harmful to aquatic life	X	X	X	X
Polyester	Harmful to aquatic life		X	X	X
PEI	Harmful if swallowed, harmful to aquatic life	X	X	X	X
Rayon	Under evaluation (pre-registered)	X		X	X
UA	No hazard reported	X			

4. Discussion

The ingestion of microplastics by fish from both freshwater and especially marine environments has been widely documented (Wang et al., 2020). Despite of current studies about microplastics presence and effects have been mainly focused on marine fishes, while data for freshwater species are still insufficient, the evidence of microplastics ingestion has been reported in >150 fish species from both freshwater and marine systems (Jabeen et al., 2017). A review by Wang et al. (2020) concludes that microplastics contamination could occur in almost all types of aquatic habitats around the globe, suggesting that fishes, even with different food behaviours and from different trophic levels, among other features, are very susceptible to microplastics ingestion with ecotoxicological effects associated to MPs. With a wide range of physical and ecotoxicological implications of its own, MPs ability to adsorb persistent organic pollutants already present in water should not be forgotten, resulting in an additional threat to fish species (Batel et al., 2016).

The results of our study demonstrate that *Anguilla anguilla* juveniles entering European rivers carry MPs, being some of them dangerous to aquatic life following the European Chemical Agency. The presence of MPs in dorsal muscle, found in other species inhabiting the North Atlantic Ocean (Barboza et al., 2020), is reported in European eel for the first time. MPs have not yet been analysed in depth in fish muscle, although its presence in that tissue has already been described in different species (Akhbarizadeh et al., 2018; Barboza et al., 2020). MP particles are usually reported into the gastric tract. Their translocation to other tissues, such as liver (Avio et al., 2015; Collard et al., 2017; Jovanović et al., 2018) and muscle (Jovanović et al., 2018), is a reality (De Sales-Ribeiro et al., 2020). However, that alleged translocation – especially of large particles-is difficult to explain with the current knowledge on translocation pathways for MPs in fish (De Sales-Ribeiro et al., 2020). In any case, the relative big size of at least one of the MPs found in muscle in this study makes it difficult to cross the gastrointestinal membranes without a serious, lethal damage to the individual. Therefore, its presence in dorsal muscle suggests instead its incorporation in early developmental stages, perhaps in the Sargasso Sea where there are high levels of floating plastic (Carpenter & Smith, 1972), or during the first marine stages of the travel to the rivers (leptocephalus eels). This discovery confirmed the first departure hypothesis about marine MPs in this diadromous species, and strongly suggests that young eels are moving at least some MPs from the sea to the river.

The results obtained in eel juveniles crossing the barrier between biomes (in sea to river direction in this case) could be extrapolated to other diadromous species, not only catadromous. In this study, eel juveniles will introduce some marine MPs in the river. If they are inserted in the dorsal muscle as seen in this study, the eel will probably leave the river with them if it survives all the freshwater periods. If the eel dies in the river, these MPs of marine origin will be deposited in the river. In the opposite direction, anadromous fish juveniles contain MPs acquired in the river; see for example O'Connor et al. (2020) for brown trout, or Collicutt et al. (2019) for juvenile Chinook salmon. Those individuals will transport the MPs to the sea. Importantly, if the waters in the transition river-sea are especially polluted (as in River Cabra in this study), vulnerable juvenile smolt (= young salmonids adapting physiologically to the salinity gradient) will be exposed to an additional stress.

Our results also support river water contribution to MPs pollution of juvenile eels crossing the barrier between biomes, in accordance with our second departure hypothesis. At their arrival in front of the river they receive MPs transported by river waters that, as reported in many studies, are main sources of marine MPs (Browne et al., 2011; Lebreton et al., 2017). Since the vast majority of these MPs were not visible from the fish surface, we could reasonably assume that they were inside the individuals, likely in the gastrointestinal tract, in agreement with the ingestion of MPs by eel juveniles described by Steer et al. (2017). Therefore, as the gastrointestinal tract is especially important as entry of MP to the organism, exploring the microplastic transfer inside and between organisms into the trophic web is essential (O'Connor et al., 2020). Eels would bioconcentrate MPs, as suggested for other fish (Mattsson et al., 2017; Assas et al., 2020; Miller et al., 2020; Kim et al., 2021), because MPs density per gram was much higher in eel tissue than in water and sediments. Nevertheless, the biota magnification between the specific predator-prey interactions in MP is not expected, as is shown by modelling studies with the greatest predicted concentration in benthic macroinvertebrates and the lowest in fish species such as European eel (O'Connor et al., 2022).

Being MPs profiles in eels similar to the ones in river water - and sediments in some cases (Supplementary table 2 and Table 2), it can be safely assumed that the river provided the majority of glass eels' MPs (Jabeen et al., 2017; Parker et al., 2021). In addition, seawater near the coast could also contribute to glass eel MPs pollution, since it explained significantly the dependent variable in Nalon River (Supplementary table 3). These results together describe a situation where young eels acquire MPs from the two biomes when they are around the border between them. Moreover, some MPs analysed in this study were composed of substances that are recognized as dangerous for aquatic life (Table 3). Therefore, we could conclude that both oceanic and riverine MPs pollution are contributing to the MPs load in European eel juveniles, being serious factors of risk for this critically endangered species.

The three rivers analysed here are exposed to different pollution sources, as explained above. The largest Nalon River crosses mining zones and has industry and ports, while the much smaller Bedon and Cabra rivers are located in rural areas and would be expectedly cleaner. However, the relative MPs contamination of each river did not follow the logical expectation of higher pollution levels in Nalon River than in the other two. Actually, water, sediment and eels from Nalon River were the least MP-polluted of the three rivers; and the highest MPs concentration in glass eels was unexpectedly found in the smallest Cabra River (Supplementary table 3). Taking the results with caution for the few rivers studied, they would support Meijer et al. (2021), where small rivers contribute to MPs pollution more than it could be expected from their size. In some cases this could be explained from deficient WWTP facilities (Estahbanati & Fahrenfeld, 2016; Talvitie et al., 2017; Xu et al., 2019). Interestingly, in this river there is a WWTP very close to the mouth where glass eels are entering (Fig. 1), and sporadic malfunctioning in the retention systems, although speculative, could not be discarded. Another explanation that cannot be ruled out to interpret a higher eel pollution in Cabra River, could be related with a higher ingestion of MP in that river. Leptocephali do not eat until VIA2 stage (Casamajor et al., 2000), and some glass eels were in that stage only in Cabra River (Supplementary table 1). Thus in this river they could be already eating, and ingesting MPs, while in the other rivers they would rather acquire them via gills. However, since MPs acquisition and bioaccumulation has not been investigated in *Anguilla anguilla* yet, this explanation remains speculative.

As expected from previous studies in this (Masiá et al., 2019) and other zones (Gewert et al., 2017; Baalkhuyur et al., 2020), fibres were the most abundant class of particles recovered from all the samples and rivers, and black and blue fibres amongst them (Kumar et al., 2018; Baalkhuyur et al., 2020). Rayon was the most abundant material, as in other studies (Frias et al., 2016; Zhang et al., 2019; Zhu et al., 2020), followed by PEI that is a modified PE – also very abundant in this and other regions, especially in seawater (Baini et al., 2018; Cincinelli et al., 2021). On the other hand, the MPs profiles of the rivers were significantly different despite their relative geographical proximity, also consistently with previous studies (Masiá et al., 2019). This and the apparently persistent spatial differences in consecutive years (Fig. 5) support the need to evaluate MPs risks for diadromous species at a local scale, since they may be very different between rivers even at a short scale.

5. Conclusions

As an example of diadromous species, European glass eels entering Bay of Biscay rivers are highly polluted with MPs, some outside of the gastrointestinal tract being located in the dorsal muscle. From the location of MPs in the eels and multiple regression analysis of MPs profiles, it seems that glass eels take MPs both from the sea and from river waters, and are able to bioconcentrate them. Some of the MPs analysed are recognized as harmful for aquatic life, putting at risk the health of the critically endangered *Anguilla anguilla*. Persistent differences between rivers suggest that the prevention of riverine MPs, necessary to avoid stress in diadromous fishes, should be done at a global but also at a local scale.

Verhelst et al. (2021) identified five major actions for diadromous fish conservation: removal of migration barriers, installation of fish passages, habitat restoration, restocking, and fisheries management. Controlling emerging microplastics pollution could be added to that list, since, as seen in this study, all analysed rivers carry a considerable amount of MPs.

More specifically, since the European eel is a critically endangered species, knowing the potential health risks caused by MPs and their origin is of paramount importance. Quantifying the amount of MPs they carry across the species distribution and in different life stages, and characterizing the sources of that pollution, should be carried out. More studies are needed on this topic, in juveniles and adults of this and other diadromous species.

Since the origin of MPs present in glass eels seems to be dual (continental + seawaters), new policies should be promoted to limit the entry of MPs in marine and river waters. Examples are improving WWTPs until near 100% removal of MPs, avoiding the use of primary microplastics through bans and public education, replacing plastic by non-plastic materials such as bamboo nets, using natural paints and other actions. Some of these policies could be efficient even applied at a local level, because river-borne MPs seem to be very specific of the river and vary at a short scale.

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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Capítulo 3:

High microplastics concentration in liver is negatively associated with condition factor in the Benguela hake *Merluccius polli*.

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High microplastics concentration in liver is negatively associated with condition factor in the Benguela hake *Merluccius polli*

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Keywords: African waters; Condition Factor; DNA degradation; *Merluccius polli*; Microplastics

Abstract

Microplastics (MPs) affect both marine and terrestrial biota worldwide for their harmful effects, which range from physical cell damage to physiological deterioration. In this research, microplastics were quantified from gills, liver and muscle of demersal Benguela hakes *Merluccius polli* (n = 94), caught by commercial trawling from northwest African waters. Plastic polymers were identified using Fourier Transformed-infraRed spectroscopy (FT-iR). Fulton's *k* condition factor and the degree of DNA degradation in liver were measured. None of the individuals were free of MPs, whose concentration ranged from 0.18 particles/g in muscle to 0.6 in liver. Four hazardous polymers were identified: 2-ethoxyethylmethacrylate, polyester, polyethylene terephthalate, and poly-acrylics. MP concentration in liver was correlated negatively with the condition factor, suggesting physiological damage. Positive association of MP concentration and liver DNA degradation was explained from cell breakage during trawl hauls during decompression, suggesting an additional way of MPs harm in organisms inhabiting at great depth. This is the first report of potential MPs-driven damage in this species; more studies are recommended to understand the impact of MP pollution on demersal species.

1. Introduction

Plastic polymers have been used worldwide for the last century, for their cheap manufacture, lightness, malleability, reusability, and resistance (Andrady and Neal, 2009). The uncontrolled use and disposal of plastic has positioned it as a global problem of huge environmental impact, for the ubiquity of plastic polymers in all known ecosystems has been proven (Rochman, 2018). As it has been widely described (Zhang et al., 2021, Li et al., 2022), plastics suffer from physical-chemical degradations leading to the appearance of microplastics (MPs thereafter). Larger pieces of plastic are actively broken by many physic-chemical factors in the marine environment such as waves (Zhang et al., 2021), sunlight (Bao et al., 2022), or even the biotic pressure (Gallitelli et al., 2022). The resulting particles of this degradation are called secondary MPs when < 5 mm length (Arthur et al., 2008). MPs that are directly manufactured of this size or smaller are called primary MPs and are generally employed in personal care products and cleansers. MPs are found in different shapes, such as fibers, fragments, films, microbeads, or pellets (Ngo et al., 2019, Lorenzo-Navarro et al., 2021). Although the variety is enormous in different marine fish, blue and black fibers are the most abundant type (Hossain et al., 2019, Abidli et al., 2021, Menéndez et al., 2022).

According to Ryan et al. (2019), Carpenter and Smith (1972) reported the first evidence of MPs in an aquatic system in 1972. The occurrence of MPs in the marine environment as well as in hundreds of marine species has been widely studied all along the coasts and seas of Africa, Eurasia, Australia, and America (Kroon et al., 2018; Ita-Nagy et al., 2022; Masiá et al., 2022a, Masiá et al., 2022b; Piyawardhana et al., 2022; Bilbao-Kareaga et al., 2023), and even in the polar regions (Morgana et al., 2018, Kögel et al., 2022). For the last 50 years, thousands of studies have reported potential effects of plastics in the marine environment, many focusing on marine species ranging from plankton (Lima et al., 2015, Rodrigues et al., 2021) to big cetaceans (Fossi et al., 2012, Zhu et al., 2019a), including filter feeders (Naji et al., 2018, Expósito et al., 2022), fishes (Neves et al., 2015; Menéndez et al., 2022), or algae (Wu et al., 2019, Menendez et al., 2021), among others. MPs can be ingested by many species (Boerger et al., 2010, Nicastro et al., 2018, Markic et al., 2020, Collard and Ask, 2021). One of the dangers derived from microplastic ingestion is chemical damage. Plastics categorised as “Group 7 plastics” such as epoxy resin or polycarbonates are manufactured with Bisphenol-A (BPA) (Yang et al., 2011) which is an endocrine disruptor. Its consumption can lead to a wide range of diseases such as neonate malformations, infertility or even cancer (Vandenberg et al., 2007). Also “Group 3 plastics” (PolyVinyl Chloride – PVC) are toxic, and their intake has been reported to induce cancer and to reduce the hepatic functioning (Wagoner, 1983) as well as DNA damage (Lei et al., 2004), among other effects. Harmful effects on both animals and environment have been also documented not only for the plastics themselves but also for the compounds attached to their surfaces (Brennecke et al., 2016, Ribeiro et al., 2017, Rehse et al., 2018, Yuan et al., 2020). Plastics can retain hydrophobic compounds such as heavy metals that are frequent pollutants of aquatic ecosystems (Rainbow, 1985, Copat et al., 2013, Makedonski et al., 2017, Tian et al., 2020, Javanshir Khoei, 2022). The exposure to these elements leads to the development of different diseases and medical conditions such as mitochondrial dysfunction (Sun et al., 2022), kidney damage (Achparaki et al., 2012) or cancer (Jaishankar et al., 2014), among others. Some heavy metals can actively cause DNA damage (Hengstler et al., 2003, Zocche et al., 2010, Ngo et al., 2021). For the elements attached to MPs, or alone, Reactive Oxygen Species (ROS) metabolism modifications and even cell apoptosis (Xia et al., 2008, Thubagere and Reinhard, 2010, Chiu et al., 2015) have been reported to occur due to the presence of MPs (Avio et al., 2015, Ribeiro et al., 2017).

Besides toxicity, another risk of the ingestion of MPs is physical damage caused mechanically. To give a few examples, the obstruction of cavities and ducts by MPs can be lethal (Roman et al., 2021). At a cellular level, MPs can induce cellular breakage (Espinosa et al., 2019), and cell injury and destruction have been reported (Wang et al., 2022a, Wang et al., 2022b, Wang et al., 2022c; Manu et al., 2023). This can be due to modifications in the cellular membrane permeability and the induction of an inflammatory reaction (Deng et al., 2017). Mechanical cell destruction due to MPs has been also reported (Fleury and Baulin, 2021; Wang et al., 2022a, Wang et al., 2022b, Wang et al., 2022c). In addition, DNA damage can be induced when MPs are abundant in the tissue (Prokić´ et al., 2019; Masiá et al., 2021).

Although the presence of MPs in marine organisms has been described from the Artic (Herzke et al., 2021) to tropical and subtropical marine ecosystems (Costa and Barletta, 2015), only a few studies have focused on MP pollution in the north-western coast of Africa (Kim et al., 20188; Maaghloud et al., 2021, Maaghloud et al., 2020; Wang et al., 2022a, Wang et al., 2022b, Wang et al., 2022c). Masiá et al., 2022a, Masiá et al., 2022b alerted of the potential risk of MP pollution for African fishing resources. One of the important resources in Atlantic African waters is the Benguela hake (*Merluccius polli* Cadenat, 1950), that is abundant in the north-west coast due to the accused seasonal upwelling (Mbaye et al., 2015). Its fisheries are also of great importance for the European fleet (Rey et al., 2012). Spanish trawlers have been fishing from Morocco, Senegal and Mauritania waters for the last 40 years (FAO, 2020; Soto et al., 2022). Hakes inhabiting Atlantic (Neves et al., 2015, Cabanilles et al., 2022) and Mediterranean (Mistri et al., 2022) waters may carry a considerable MP charge. However, to our knowledge, and despite the risk of MP pollution in west African coasts (Masiá et al., 2022a, Masiá et al., 2022b), the content of MPs in Benguela hake has not been investigated yet.

In this study, we have quantified and analysed the abundance of MPs in different tissues of Benguela hake adults: muscle, which is the edible tissue in hakes; gills, that are the first contact with MPs from the water column (Guilhermino et al., 2021), and liver. MP accumulation in liver may negatively affect the health of the organisms (Yu et al., 2018), something that in fish can be reflected in a worse condition factor (Amorim et al., 2020). The status of the sampled hakes was evaluated from Fulton's *k* condition factor, and the possible damage to liver from the degree of DNA degradation (Masiá et al., 2021). From the negative association between MP charge and fish status, the starting hypothesis will be that the individuals with more MPs in liver would exhibit a worse condition factor and a higher level of DNA degradation.

2. Materials and methods

2.1. Species in study and samples analysed.

Merluccius polli is an Actinopterygii from the Merlucciidae family. Its natural distribution ranges from southern Morocco (NW Africa, 28°N) (Manchih et al., 2018) to the Northern coast of Namibia (SW Africa, 18.30°S)(Lloris et al., 2005); according to the International Union for the Conservation of Nature (IUCN), it is catalogued as Least Concern (https://www.iucnredlist.org/es/species/15522226/15603610). It is sympatric to Senegalese hake (*Merluccius senegalensis*), although the Benguela hake can occupy a greater range of pressures and temperatures (Fernández-Peralta et al., 2011). Benguela hake preys upon small fishes, little squids, and shrimps (FAO, 1990), with some cannibalistic behaviour in adults, depending on the availability of food and resources (Kilongo and Mehl, 1997). Its exploitation has increased exponentially for the last 20 years. In 2006 approximately 9000 tonnes of Benguela hakes were caught, while in 2018 the amount rose to 20,000 tonnes according to FAO (2019).

A total of 94 commercial individuals, in whole and fresh (kept in ice), were kindly provided by the Cádiz Fish Market (Lonja de Cádiz). The samples had been fished by trawling in the 34.1.3 FAO Fishing Area (Fig. 1), in the Western coast of Africa. Since they were caught by commercial fleet for selling, not sampled in purpose for this study, an ethic statement is not needed for this research.

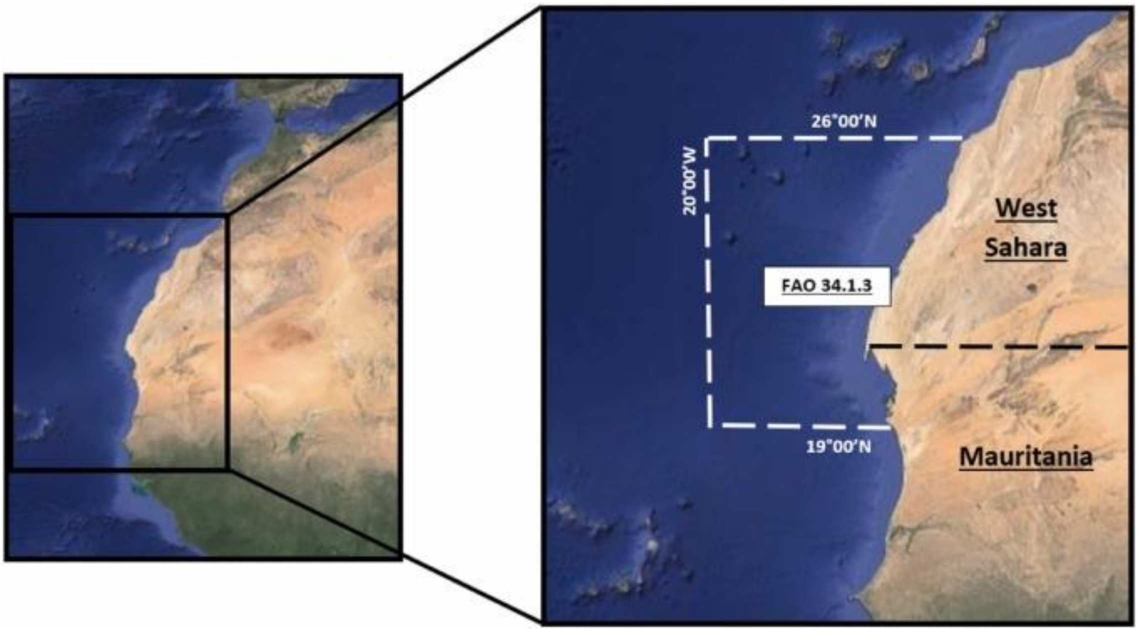

Fig. 1. Fishing area where the individuals analysed in this study were caught from commercial trawling.

Individuals were measured (standard length was taken), weighted, and dissected for the recovery of muscle, liver, and gills. Samples were labelled and stored in the freezer until processing. For all the samples, approximately 20 g of dorsolateral muscle, 4 gill arches (the same side as the muscle) and 5 g of liver were processed.

2.2. Molecular identification by PCR-RFLPs

All individuals came labelled as *Merluccius polli* from the supplier. Additionally, the species assignation was checked with PCR-RFLPs, as this species is captured in mixed fisheries with its sympatric species (*Merluccius senegalensis)* and both species are often mislabelled (Blanco-Fernandez et al., 2022). DNA was extracted using Chelex®, following the protocol developed by Estoup et al. (1996). The mitochondrial control region was selected as target to discriminate between both species, since it is known to be a variable region with polymorphisms between the different species of the *Merluccius* genus (Machado-Schiaffino et al., 2008). A fragment of control region (450pb) was amplified using the primers MmerHk01 and MmerHk02 developed by Lundy et al. (2000). Amplifications were performed in a final volume of 40 µL using each primer in a final concentration of 0.5 µM, dNTPs at 0.25 mM, $MgCl_2$ at 1.5 mM, Green GoTaq® G2 Flexi Buffer 1x, and DNApol GoTaq® G2 Flexi DNA Polymerase at 0.0375 U/µL. PCR conditions were set to an initial denaturing step of 5' at 95 °C, followed by 35 cycles consisting on denaturing for 30" at 95 °C, annealing for 30" at 55 °C and extension for 30" at 72 °C, and then a final extension step at 72 °C for 15'.

The restriction enzyme BseGI (BTSCI) was selected to generate RFLPs after validation. The enzyme was choosen after searching for a polymorphism that would allow for a differential cut between *M. polli* and *M. senegalensis*. This search was carried out using *in silico* simulator NEBcutter v3 (https://nc3.neb.com/NEBcutter/) to locate the target sequences for commercial restriction enzymes. The enzyme BtsCI would digest the amplicon in two fragments of 216 bp and 226 bp respectively for *M. polli*, while amplicons from *M. senegalensis* remained undigested (442 bp fragment). For its *in silico* validation, 93 sequences corresponding to haplotypes of 806 individuals of both species (60 sequences belonging to *M. polli* and 33 to *M. senegalensis*) were taken from Blanco-Fernandez et al. (2022) (GenBank accession numbers from MZ703314 to MZ703406). All sequences were aligned using MUSCLE in BioEdit and the target position was checked to see whether the polymorphism was maintained along all samples of both species. Out of the 806, only one specimen of *M. polli* did not present the polymorphism that would allow for the digestion, setting an overall error of 0.12 %. After the *in silico* validation, a digestion was performed with known samples from *M. polli* and *M. senegalensis*. For the digestion, we added 10 µL of the previous PCR as template, BseGI(BTSCI) at 0.67 U/µL, and Thermo Scientific™ 1x Buffer Tango in a final volume of 30 µL. The reaction was left incubating at 55 °C for 3 h. Then, results were visualised in agarose gel 2 % stained with 2.5 µL SimpleSafe (Eurx) and run at 120 V for 30 min. Once this methodology was established, the same procedure was applied to the samples of this study. Additionally, 30 randomly chosen from the 94 samples were Sanger sequenced for further verification.

2.3. Microplastic extraction and quantification

First, tissues were digested using 10 % KOH (Thermo Fisher Scientific®). The proportion of reagent per gram of tissue was 1:5 (w/v) for muscle, proportion 1:10 for gills, and 1:50 for liver. Tissue digestion was carried out at 40 °C for 48 h in glass jars covered with aluminium foil to avoid contamination. Blanks consisting of 100 mL of KOH were placed in every oven together with the samples to control for possible contamination during the lab work.

After digestion, two phases were obtained from liver samples: the lower, which is the digested material, and the top one, which is non-digested fat. Jars were refilled with 100 mL of filtered neutral laboratory soap (Labbox, Spain) per 100 mL of digestion to disaggregate and dissolve the fat, for further filtration. This process takes between one and two hours with periodical manual shaking to detach the fat from jar walls. For gill and muscle samples, and blanks, 100 mL of filtered distilled water were added and the same process was followed (1–2 h, manual shaking) to homogenise the process across samples for the control of procedural contaminations.

Finally, all digestions were filtered through 1.2 µm pore size glass microfiber filters (Whatman GF/C, 47 mm diameter) that were allowed to dry in glass petri dishes for 48 h before MP counting. Once dried, filters' surfaces were observed individually under the microscope and all potential plastic particles were counted following Hidalgo-Ruz et al. (2012). Due to the colour and morphology of the glass microfiber filters, special attention was paid to white and transparent particles. A heated needle was approached to potential particles to confirm they were of plastic (plastic bends when heated while organic matter and glass do not). Counting and visual identification were carried out under a Leica 2000 stereomicroscope at 40x magnification (Masiá et al., 2019, Menéndez et al., 2022). Only < 5 mm particles were considered for further analysis.

MPs were first classed by shape as in previous studies (Kumar et al., 2018; Hossain et al., 2019; Masiá et al., 2022a, Wang et al., 2022a, Wang et al., 2022b, Wang et al., 2022c). Three main groups were identified: fibers (elongated, uniform colouration and mostly cylindrical), fragments (irregular shapes, generally with sharp angles), and microbeads (plastic spheres, after checking for possible misidentification with small eggs) (Neves et al., 2015, Güven et al., 2017, Yin et al., 2022). Colour was also recorded (Zhu et al., 2019b, Guilhermino et al., 2021).

A 16 % of putative plastic particles ($n = 120$), roughly representative of all the shapes and colours found in the samples, were analysed by Fourier Transformed infrared spectroscopy (FT-iR) (Uurasjärvi et al., 2021) in the Autonomous University of Madrid. They were picked from the petri dishes under laminar flow cabin to prevent airborne contamination. The analyses were performed using a wavelength between 4000 and 500 cm^{-1} and a germanium glass, Varian 620-IR and Varian 670-IR. Results with a bibliographic search score over 60 % were used. The potential toxicity of the compounds for the aquatic life and/or for humans was checked in the European Chemicals Agency (ECHA; https://echa.europa.eu/es/home, accessed March 2023).

2.4. Contamination control

To control for potential contamination from airborne particles, all procedures were performed into a semi-closed laminar flow cabinet. A cotton white lab coat was constantly worn by the researchers as well as nitrile gloves. All materials in contact with the samples (scissors, tweezers, glass jars…) and implicated in the filtering (vacuum pump) were previously rinsed with filtered distilled water. Distilled water was filtered through 0.22 µm pore size PES filters (PALL Corporation®, 47 mm diameter). Also, the laboratory soap was filtered in the same conditions, while KOH was filtered through 1.2 µm pore size glass microfiber filters (Whatman GF/C, 47 mm diameter). Filters were stored in clean petri dishes which remained closed until the particle's selection for the chemical analysis.

2.5. Genomic DNA extraction and DNA degradation analyses

DNA was extracted from the liver of 46 individuals (48.9 % of the total sample) representing all the range of MPs concentrations found in this study. A small piece of tissue (≤ 25 mg) was disaggregated mechanically, and the genomic DNA was extracted with a commercial kit (DNeasy Blood & Tissue kit, Qiagen, Hilden, Germany), following the manufacturer's instructions. Extractions were quantified using a Qubit 4 Fluorimeter (Thermo Fisher Scientific, Inc). The electrophoresis was performed based on Mičić et al. (2002) and Masiá et al. (2021): 30 ng of DNA (variable µL of each extraction) was run in a 1.3 % agarose gel for 2 h at 90 mV. The level of DNA degradation was categorised in four groups (G1-G4) following Masiá et al. (2021) (Supplementary Figure 1).

2.6. Condition factor

Fulton's *k* condition factor of each individual (Fulton, 1904) was calculated following the equation:K=100xWL3

Being W the full body weight in grams and L the standard length in cm (Froese, 2006).

2.7. Statistical analysis

Differences between groups of samples for variables distributed in discrete categories (for example proportion of MPs of different colours or shapes) were tested using contingency chi-square analysis.

Quantitative data like MPs/g were compared between groups of samples (e.g., tissues) using one-way ANOVA, after checking normality from Shapiro-Wilk test and homoscedasticity from Breusch-Pagan test. Post-hoc Tukey's pairwise tests were performed after significant ANOVAs.

Multiple linear regression analysis was applied for condition factor as dependent variable and MP concentration in the different tissues as independent variables, to infer if any tissue pollution could be a predictor of the hake condition. Pairwise Pearson's correlation tests were run to check for associations between variables, e.g., MP concentration and DNA degradation.

Statistical results were interpreted under a 95 % confidence interval (standard significance threshold $p < 0.05$), applying Bonferroni correction for multiple comparison when needed. Statistical analysis was done in PAST free Software V.2.17 (Hammer et al., 2001).

3. Results

3.1. Microplastics content in Benguela hake tissues

BseGI PCR-RFLPs successfully assigned all individuals as *Merluccius polli*, as had been reported. A total of 747 particles identified visually as putative MPs were obtained from the 94 Benguela hakes analysed (Supplementary Table 1). All the individuals had at least one tissue with MPs, from 80 % of gill to 94 % of muscle samples; for the shape, the majority of particles were fibers, a few fragments, and only one microbead in gills (Table 1).

Table 1. Overview of MPs identified from each tissue. N: number of particles identified.

Tissue	% individuals affected	Mean MPs/g (variance)	Fibers	Fragments	Microbeads	N
Gills	**80 %**	**0.517 (0.168)**	**95.4 %**	**4 %**	**0.6 %**	**175**
Liver	**83 %**	**0.599 (0.355)**	**98.2 %**	**1.8 %**	**0 %**	**224**
Muscle	**94 %**	**0.181 (0.012)**	**96.55 %**	**3.45 %**	**0 %**	**348**

Blanks run all over the experimental work showed a concentration of 0.003 MPs/g, which is two orders of magnitude lower than the tissue samples (see Table 1). Thus, significant procedural contamination could be discarded.

The relative MP load was higher in livers and gills than in muscle (Table 1). The difference among tissues was highly significant (ANOVA with $F_{(2279)} = 25.81$, $p < 0.0001$). Post-hoc Tukey's test showed significant difference between muscle and liver ($t = 6.68$, $p < 0.0001$) as well as between muscle and gills ($t = 7.67$, $p < 0.0001$).

From the relative frequency of different types of particles, MPs were classed for analysis in six groups: blue, black, transparent, and other (including reddish, green, orange, and purple) fibers, blue fragments, and transparent microbeads. Fig. 2 shows the profile of the particles recovered from the three tissues. The majority of MPs were black, followed by blue, transparent fibers, fibers of other colours, blue fragments and one transparent microbead in a gill. The global contingency chi-square was statistically significant (χ^2=28.87, d.f.=10, $p = 0.016$; Cramer's V = 0.12) due to the difference between muscle and liver (χ^2=14.01, d.f.=4, $p = 0.007$; Cramer's V = 0.16); the rest of comparisons between tissues were not significant (data not shown). Livers contained more black and fewer blue fibers than muscle samples (Fig. 2).

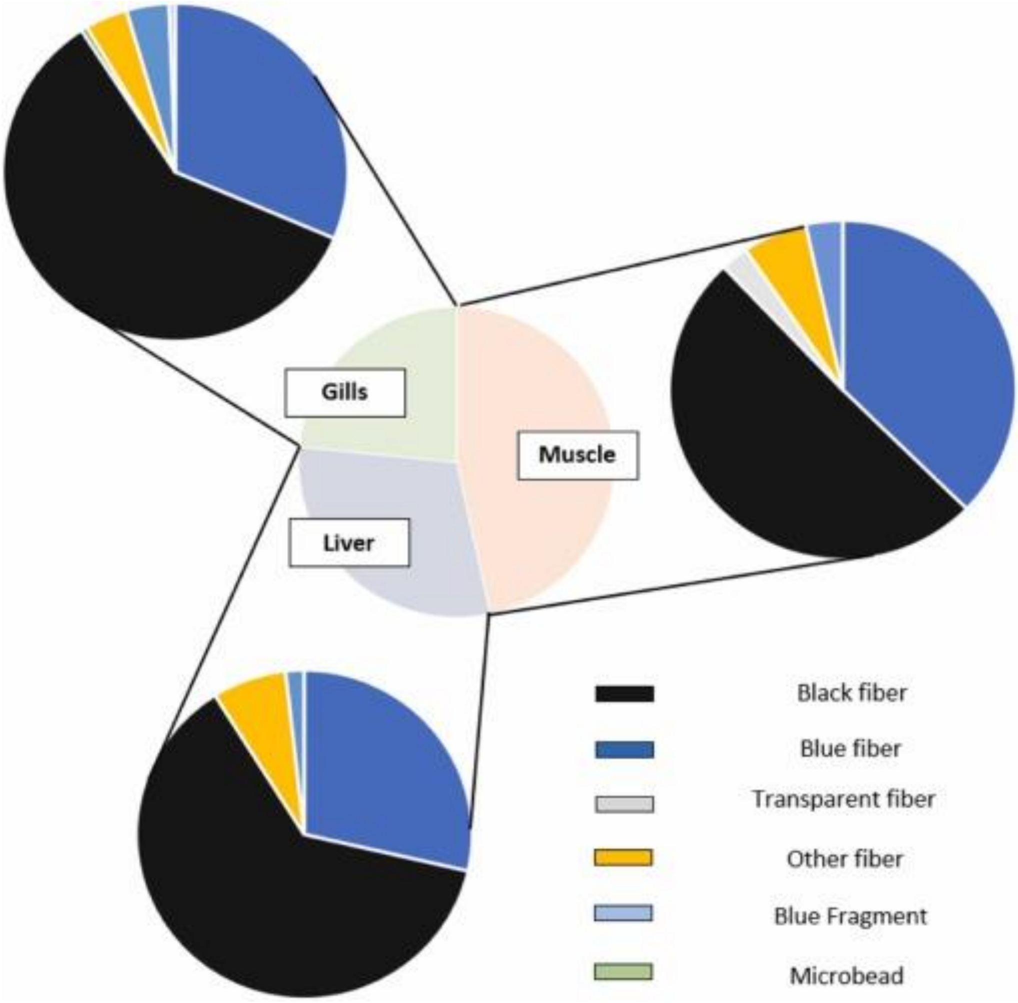

Fig.2. Colour and shape of the microparticles analysed from muscle, gills and liver.

FT-iR analysis was done on 53 particles recovered from muscle, 39 from liver and 28 from gill tissues. Seven different compounds were identified (Fig. 3): Rayon (55 %), PEI – Polyethyleneimine cellulose (9.17 %), PET-Polyethylene terephthalate (7.5 %), Polyester (5.83 %), PAN/PAA – Polyacrylenitrile/Polyacrylic acid (2.5%) and 2-ethoxyethyl methacrylate (0.83 %). The remaining 19.18 % particles were of natural compounds such as cellulose. Excluding cellulose, contingency chi-square analysis did not show significant differences between tissues (χ^2=12.461, d.f.=10, p = 0.255).

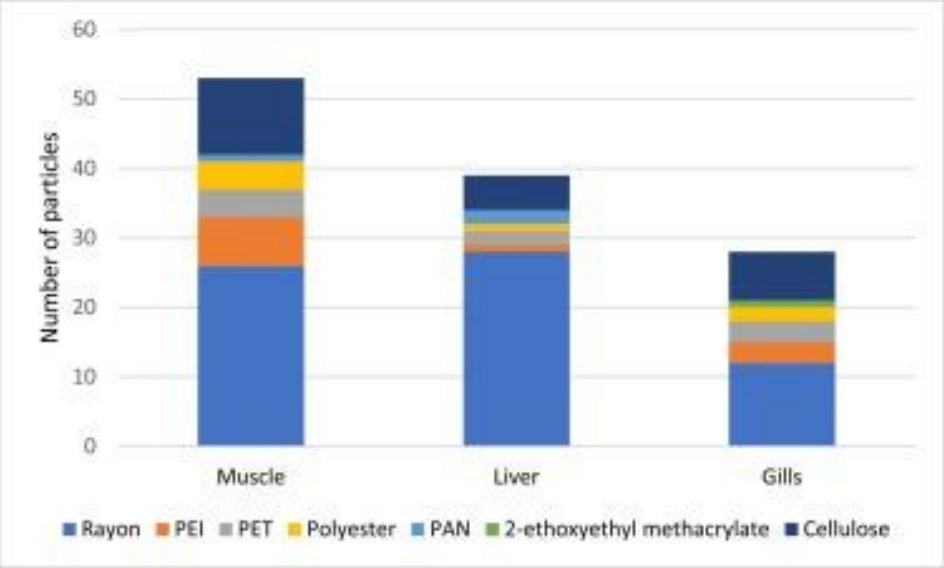

Fig. 3. Proportion of particles of different compounds in the Benguela hake tissues analysed.

From the ECHA, four of those compounds are harmful for living beings (Table 2); therefore, Benguela hake is expected to be affected by those MPs.

Table 2. Potential hazard of the compounds identified in this study, according to the European Chemical Agency. Rayon and PET are under research.

Compound	Harmful to aquatic life	Harmful if swallowed	Irritative	Affects fertility and unborn child
Rayon	Pre-registered, no information available			
PEI	X	X		
PET		Pre-registered, no information available		
Polyester	X			
PAN/PAA	X		X	
2-Ethoxyethylmethacrylate			X	X

3.2. Condition factor

Fulton’s ‘*k*’ condition factor varied widely in the samples analysed, ranging between 0.401 and 1.114 g/cm^3 with an average of 0.886 (SD 0.094). The modal class was the group with *k* [0.839–0.912] (Fig. 4).

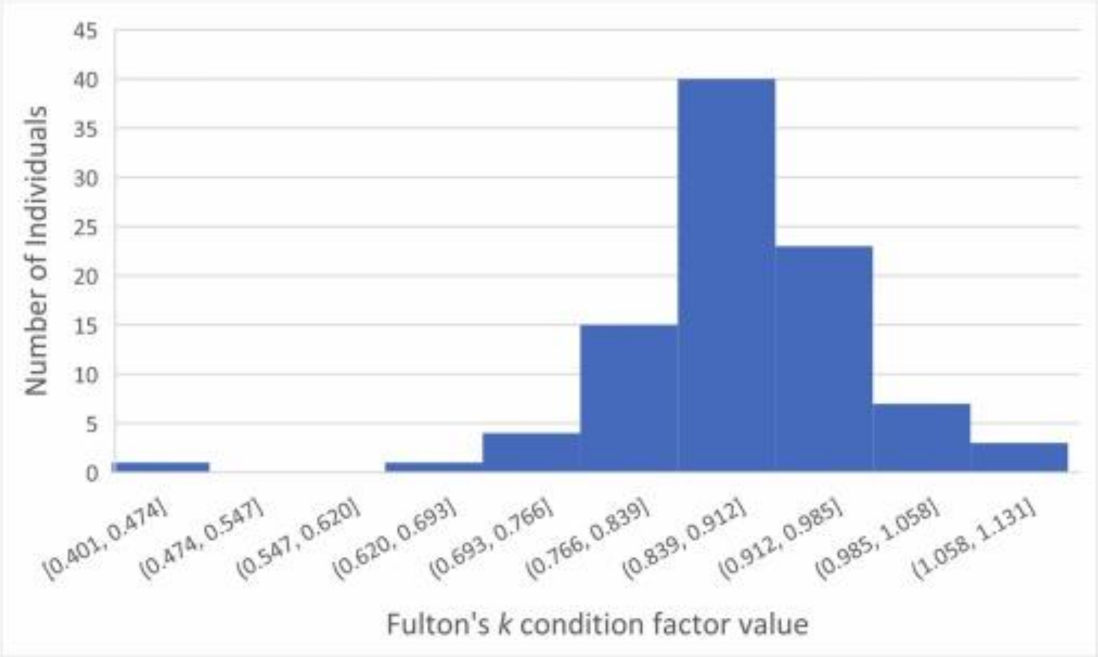

Fig. 4. Distribution of the analysed Benguela hakes by Fulton's k condition factor.

From multiple linear regression, with the condition factor as dependent and the concentration of MPs/g in different tissues as independent variables, only the MPs concentration in liver significantly predicted the condition factor (Table 3). The regression slope was significantly negative (r = −0.234, *p* = 0.024).

Table 3. Linear regression results with Fulton's k Condition Factor as dependent variable and the concentration of MPs in different tissues as independent variables. SE, standard error.

	Coefficient	**SE**	**t**	***p***	**r^2**
Constant	0.92	0.023	39.767	0.000	
Muscle MP	-0.045	0.088	0.515	0.608	0.003
Liver MP	-0.037	0.016	22.926	0.024	0.055
Gills MP	-0.006	0.021	0.244	0.807	0.001

3.3. DNA degradation in liver

The individual results obtained for liver DNA quantification and the assigned degree of degradation (Masiá et al., 2021) are in the Supplementary Table 2. Mean DNA concentration of the 46 liver samples analysed was 18.7 (SD 12.6) ng/μL. The average level of DNA degradation was 2.65 (SD 0.90) over a maximum of four, implying that quite fragmented DNA was found for many individuals (Fig. 5). Only six individuals exhibited a clear band of large genomic DNA (group G1, not degraded), while seven individuals yielded very small DNA fragments (group G4) (Fig. 5).

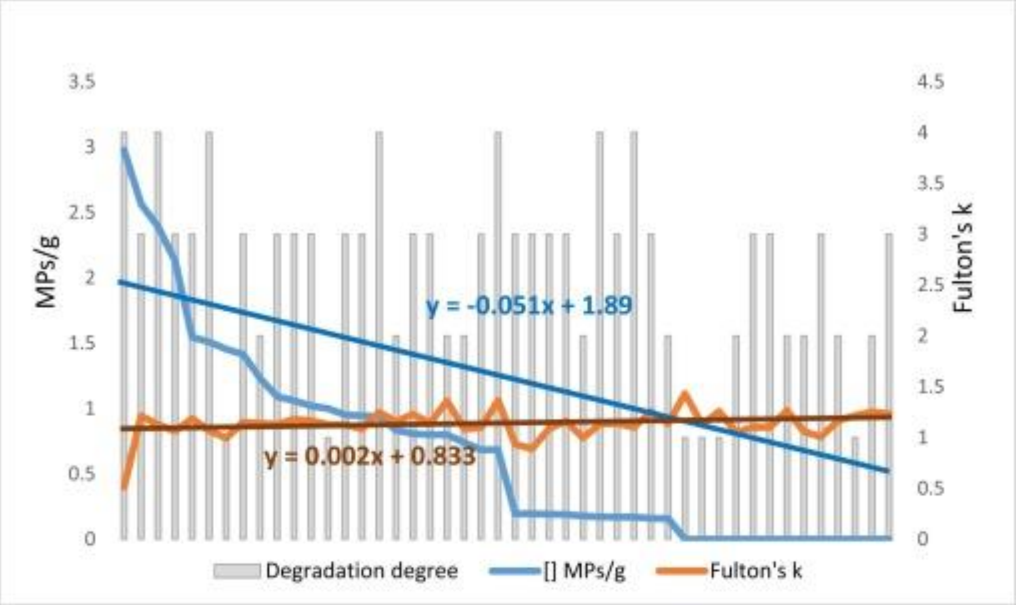

Fig. 5. Relation between the concentration of MPs/g (blue irregular line and blue trend line), Fulton's k Condition Factor (orange irregular line and brown trend line) and DNA Degradation Degree (grey columns, scores 1–4). The equation of trend lines is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As expected, a positive significant correlation was found between the estimated DNA degradation and the concentration of MPs/g in liver ($r = 0.35$, $p = 0.015$; see blue trend line of MPs/g in Fig. 5), suggesting that MPs may contribute to degrade DNA. To explore this effect further, we divided the samples in three groups: one with no MPs found in liver ($n = 13$; mean DNA degradation 2.0, SD=0.82), another with MP concentrations up to 1 MPs/g ($n = 20$; mean DNA degradation 2.95, SD = 0.67), and another with ≥ 1 MPs/g ($n = 13$; mean DNA degradation 2.85, SD =0.99). One-way ANOVA for DNA degradation was highly significant ($F_{2,45} = 5.85$, $p = 0.005$), and the post-hoc Tukey's test showed that the group of hakes without MPs in liver had significantly less degraded DNA than the group with between 0 and 1 MPs/g ($t = 4.47$, $p = 0.01$), and also less than the group with more than 1 MPs/g ($t = 3.98$, $p = 0.02$). The difference between the two groups with MPs in liver was not significant ($t = 0.49$, $p = 0.9$ n.s.).

While the correlation between the condition factor and the concentration of MPs in liver in this subsample was significantly negative ($r = -0.32$, $p = 0.03$), as it was in the whole sample of 94 hakes (see results in 3.2 above), the correlation between the condition factor and liver DNA degradation was not significant ($r = -0.23$, $p = 0.118$); a quite flat brown trend line can be observed in Fig. 5. This suggests that, unlike liver MPs content, the level of DNA degradation found in this assay is not related with the physiological condition of hakes (see below).

4. Discussion

To the best of our knowledge, this is the first study that evidences the occurrence of MPs in Benguela Hake (100 % prevalence), some of compounds identified as harmful to aquatic life. From these results, we could expect the most MP-polluted hakes exhibit a poorer physiological condition, as found in other fish (Amorim et al., 2020), especially if MPs are in liver (Yu et al., 2018). This was confirmed from a significant negative association between MP content in liver and hake condition factor and would suggest that MP pollution is endangering this important fishing resource. In our study, the MPs in muscle were statistically different from those found in liver and gills. This may suggest that the MPs that reach the liver and the gills are similar; more studies should be carried out to confirm this relationship. As in other fish (Guilhermino et al., 2021), liver exhibited the highest MP concentration, supporting its suggested role of bioaccumulation of these pollutants (Lu et al., 2016, Collard et al., 2017, Yu et al., 2018). The accumulation of plastic particles in the liver may affect the health status of the organism, which is suggested in our study by negative correlation between MPs content and condition factor. Table 3

The mechanisms linking MPs in liver and worse physiological conditions are probably a combination of chemical and mechanical damage. Harmful polymers (Table 2) surely interfere with tissue functioning. DNA damage is known as an effective indicator of environmental ecotoxicity (Dimitriadi et al., 2021), and a higher amount of MPs may lead to higher DNA damage (Shen et al., 2022). Moreover, the association between MP content and DNA degradation, found here for the first time in fish, would suggest a higher level of cell breakage in liver in the individuals with MPs. It can be interpreted as a signal of broken cells where DNA is no longer protected inside the nucleus wall, because the presence of MPs in soft tissues might lead to the cell and/or DNA breakdown (Wright et al., 2013, Espinosa et al., 2019, Masiá et al., 2021, Sobhani et al., 2021). Indeed, highly degraded liver DNA in our study does not mean that there were no integer cells when the hakes were alive (they could not survive without functional liver cells); it could be attributed to a higher cell breakage in livers with MPs during the fishing process instead. For that, the level of DNA degradation found in this assay is not related with the physiological condition of hakes. *Merluccius* *polli* is a demersal/bathydemersal fish, so its body is naturally subjected to high pressures (depth down to 1000 m), and the samples here analysed were obtained from commercial trawling. Fish internal organs are often damaged during haul backs for rapid decompression (Suuronen, 2005); likely the presence of MPs contributed to mechanical damage of the organs under decompression, facilitating cell breakage (Espinosa et al., 2019; Masiá et al., 2022a, Wang et al., 2022a, Wang et al., 2022b, Wang et al., 2022c) and subsequent DNA degradation (Masiá et al., 2021). Therefore, as it was showed in the results section, the level of DNA degradation found in this assay is not related to the physiological condition of the hake.

Regarding the prevalence and quantity of MPs, the results showed that all the hakes analysed contained MPs at least in one of the three tissues; which is relatively high if compared with other *Merluccius* hakes from Gulf of Cadiz (Bellas et al., 2016) or the South Pacific (Pozo et al., 2019), with the exception of a few *M. merluccius* individuals from the Cantabrian Sea that were highly contaminated with MPs (Cabanilles et al., 2022). MPs concentrations were also higher than those reported for hakes in Portugal (Neves et al., 2015), in the Mediterranean (Anastasopoulou et al., 2013; Giani et al., 2019; Mancuso et al., 2019; Bošković et al., 2022; Mistri et al., 2022) or in Newfoundland (Liboiron et al., 2018); similar or slightly higher than those found in South African hakes (Sparks and Immelman, 2020), and lower than MP content in Pacific *M. productus* from Monterey Bay (Hamilton et al., 2021). In general we could say that these hakes are relatively very polluted, at least in comparison with hakes from other regions; however, it should be noted that the comparison is not straightforward because the majority of studies on hakes have analysed MPs in the gastrointestinal tract, while in our study we analysed individual's tissues.

Compared with other marine fish of Northwest African waters, our data on hake would be also relatively higher than those reported for pelagic species (Maaghloud et al., 2020, Maaghloud et al., 2021; Sánchez-Almeida et al., 2022), except for chub mackerel *Scomber colias* from Canary Islands (Herrera et al., 2019); to be noted again that these data are from the gastrointestinal tract. Murphy et al. (2017) found more MPs in demersal than in pelagic fish; perhaps pelagic fish are less exposed to plastic debris that accumulates near shores, flocculates and deposits over the seafloor. According with the type of MPs, our results were in concordance with previous data of MPs in marine species that are principally black and blue fibers (Hossain et al., 2019, Abidli et al., 2021, Menéndez et al., 2022). In this study, we found a majority of the same MP types. Fibers are, due to their morphology and typology, the least retained in wastewater treatment plants WWTPs (Ngo et al., 2019, Masiá et al., 2020) and are the most likely type of particle obtained from the degradation of fishing gears and nets (Montarsolo et al., 2018, Wright et al., 2021) or released from clothes (De Falco et al., 2019). Also, dark particles tend to be more consumed by fishes for their confusion with plankton (Ma et al., 2020). It is worth noting that hakes are carnivorous so many MPs might have reached the animal by trophic transfer (Au et al., 2017, Carbery et al., 2018).

Masiá et al., 2022a, Masiá et al., 2022b found in their meta-analysis that fish from Northwest Africa were relatively little polluted with MPs, but these new data on hakes would reveal more contamination than expected. As explained above, the majority of studies from this area were on pelagic fish, while Benguela hake is demersal and would be more exposed to MPs (Murphy et al., 2017). Another explanation could be a recent accumulation of MPs transported by currents, as it happens in Japanese waters (Iwasaki et al., 2017). According to NOAA (https://nowcoast.noaa.gov/ Last accessed February, 2023) the main currents that run through the sampling area have their origin in the Strait of Gibraltar and have North-south directionality. High MP pollution in fish has been reported from different areas of the Mediterranean Sea (Akhbarizadeh et al., 2019; Masiá et al., 2022a, Masiá et al., 2022b). Recent studies (Akarsu et al., 2020, Fytianos et al., 2021, Pedrotti et al., 2021) have revealed a large amount of MPs reaching the Mediterranean Sea after escaping the retention systems of WWTPs that are sources of MPs in the aquatic environment (Sun et al., 2019, Liu et al., 2021). Therefore, it is not unreasonable to consider the Mediterranean Sea as a potential source of pollution for the waters of northwest Africa. This does not exclude other MP sources like rivers (Jiang et al., 2019, Kataoka et al., 2019), or airborne plastic particles (Allen et al., 2021).

As a final remark, the results found in this study suggest MPs pose an additional threat to demersal fish. According to the OECD, about 6.1 Mega Tonnes of MPs were released to the aquatic environments in 2019, leading to an historical accumulation of 30 Mt in the oceans (OECD, https://www.oecd-ilibrary.org/sites/de747aef-en/index.html?itemId=/content/publication/de747aef-en). The only way to stop the deterioration of these valued fishing resources is to reduce the human dependence on plastic while improving plastic management.

5. Conclusions

The Benguela Hake was used here as a representative of the marine top-predators from the North-West coast of Africa. Multiple plastic polymers were found from all the individuals studied in, at least, one tissue analysed (showing a high prevalence of black and blue fibers). While most of the particles have not been reported as dangerous for the aquatic life or the human consumer (rayon was the mostly found polymer), a few compounds (in minor quantities) recognised as hazardous, were also identified.

The gills and the liver, as a direct entrance for MPs in the organisms, showed a high and similar number of particles per gram of tissue. Otherwise, the muscle, studied as an edible tissue by the potential human consumer, showed a lower but still warning presence of plastics. Once the presence and abundance of MPs was studied, their biological harmful effect has been proven in the liver, which acts as an active bio-accumulator of MPs. In the liver, the increase on the number of MPs per gram of tissue is translated into a negative effect in both the physiological condition and the DNA integrity (the higher MP concentration, the lower Condition Factor, and the higher DNA degradation, respectively).

The Benguela hake is now included in a long list of species affected by microplastics in the African coast, but a short list when we focus on the northwest coast, where not enough studies have been carried out yet. Their demersal distribution as well as their environmental importance might be taken as a wake-up call for further analyses. The identification of the preys, the potential trophic transfer as well as the study of the waters where the Benguela hake inhabits might be enough for the correct implementation of management measurements that avoid or, at least, limit the arrival of a large number of pollutants in the area.

New policies aimed at responsible waste management (land and water waste) as well as the exhaustive limitation of the distribution of single-use plastics should be a priority to preserve a balanced health status of the seas. These actions together with a proper public warning and population education must be carried out in an efficient manner to protect marine ecosystems and, therefore, human health.

CRediT authorship contribution statement

Daniel Menéndez: Methodology, Investigation, Software, Writing– original draft. **Carmen Blanco-Fernandez:** Investigation, Writing – review & editing. **Gonzalo Machado-Schiaffino:** Visualization, Writing – review & editing. **Alba Ardura:** Supervision, Visualization, Writing – review & editing. **Eva Garcia-Vazquez**: Conceptualization, Data curation, Visualization, Supervision, Writing – review & editing.

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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Discusión

1. Novedad de esta Tesis en un campo en auge

Esta Tesis tiene su origen en la necesidad de cubrir nuevas especies y regiones en la investigación global sobre MPs. En primer lugar, se han analizado dos nuevas especies como portadoras y afectadas por MPs en aguas del Atlántico asturianas y africanas, en las cuales existe una escasez de datos significativa. Hasta el momento, esta es la segunda Tesis doctoral en el área de los MPs que desarrolla sus investigaciones en la costa asturiana y uno de los pocos estudios localizados en la costa noroeste de África, continente poco estudiado hasta el momento (Masiá et al., 2022). Hasta el año 2019, los estudios de basura marina en Asturias estaban orientados hacia los macroplásticos y otros residuos macroscópicos (Rayón-Viña et al., 2018; Rayón-Viña et al., 2019). En el año 2019 vio la luz el primer estudio que ponía de manifiesto la presencia de MPs en las costas asturianas (Masiá et al., 2019), lo que funcionó como impulso para dar comienzo a un crecimiento notable en el número de investigaciones relacionadas en la zona.

Los resultados aportados en la presente Tesis muestran, en primer lugar, una serie de prioridades de investigación en el campo de las algas gelificantes afectadas por MPs. Estas algas son ampliamente utilizadas en industria para la producción de alimentos, medicamentos e incluso cosméticos (Athanassiadis et al., 2009; Joshi et al., 2018; Jayakodi et al., 2022). Debido a su creciente demanda y consumo, es necesario profundizar en los procesos de recogida y acumulación de MPs durante el procesamiento de estas algas, así como en la producción de agentes gelificantes, investigación aún en vías de crecimiento. Por otra parte, el caso de la anguila europea destaca debido a su estatus de especie protegida y en peligro crítico de extinción. Tras un número limitado de investigaciones sobre microplásticos en especies diádromas (Collicutt et al., 2019; Siddiquie et al., 2022), el estudio publicado en la presente Tesis se puede considerar como el primer análisis en profundidad de esta especie. Resultando así en la identificación del mar como una fuente de MPs para las aguas continentales con las especies migratorias como vectores. También pone de manifiesto la problemática que entrañan los MPs para la viabilidad de esta especie catádroma, cuya explotación pesquera está estrictamente regulada. Finalmente, pese a la amplia documentación existente en lo relativo al contenido de MPs en la merluza europea (*Merluccius merluccius*) (Neves et al., 2015; Mancuso et al., 2019; Cabanilles et al., 2022), el resto de las especies de merluza distribuidas por el planeta no han gozado del interés debido, pese a ser comúnmente explotadas para consumo humano. Aquí se presenta la primera evidencia del impacto de MPs sobre la merluza negra o de Benguela (*Merluccius polli*), la cual es capturada en las costas noroeste de África y comercializada en Europa, además de consumirse localmente. Las especies aquí mencionadas pertenecen a diferentes niveles tróficos, aunque no forman parte de una misma cadena trófica (unos no depredan a los otros).

A continuación, se procederá a discutir los distintos aspectos resultantes del desarrollo de esta Tesis de manera más detallada. Se comenzará por las características generales de los microplásticos atribuibles a toda la investigación, para, a continuación, discutir cada uno de los capítulos individualmente.

1.1. Características de las partículas identificadas

1.1.1. Físicas

En todas las especies y muestras estudiadas, las partículas más abundantes fueron las fibras, oscilando entre el 98.58% en las aguas del río Cabra y el 76.08% en aguas cantábricas de mar abierto paralelas a la costa asturiana. Tal y como indican Sol y col. (2020), las fibras de plástico son las partículas de más difícil retención en las plantas de tratamiento de aguas debido a su morfología. Otros investigadores, como Ngo y col. (2019) o Long y col. (2019), confirman este dato en otras regiones, lo que posiciona a las fibras como la forma de MPs más abundante que llega a los mares a través de su principal foco de contaminación: los ríos. De la misma manera, las coloraciones más frecuentes fueron las más oscuras: negras y azules. Este hecho confirma los resultados de Zhao y col. (2022), quienes indicaron que cuanto más oscuro es el color del compuesto hay una menor incidencia de los rayos UV y, por ende, menor fotodegradación (menor fragmentación por exposición a la luz). Por esto, los MPs de colores oscuros permanecen más tiempo en el agua antes de ser completamente degradados.

Es importante resaltar que los peces ingieren frecuentemente las fibras azules y negras porque las confunden con elementos de su dieta habitual (Ory et al., 2017; Compa et al., 2018; Ma et al., 2020), por lo que resulta lógico encontrar muchas de estas fibras en las merluzas. Los tipos de MPs encontrados en la presente Tesis en especies de la costa asturiana, así como los correspondientes a la costa africana, son similares a los publicados para otras especies acuáticas marinas (Cabanilles et al., 2022: Janssens & García-Vázquez, 2022; Solomando et al., 2022; Bilbao-Kareaga et al., 2023). Estos resultados se apartan ligeramente de los tipos encontrados en heces de especies avícolas costeras del Cantábrico donde las fibras negras son mucho menos abundantes que las blancas, algo explicado igualmente a partir de su dieta (Masiá et al., 2019).

1.1.2. Químicas

El análisis químico realizado mediante espectroscopia infrarroja (FT-iR) ha mostrado, en todos los casos, una mayoría clara del rayón como polímero más abundante. Este compuesto artificial empleado en tejidos no naturales se compone de viscosa, un líquido orgánico que puede ser moldeado y trabajado igual que un plástico. Actualmente, según la ECHA, el rayón se encuentra como pre-registrado, sin indicarse por tanto efectos adversos asociados a su consumo y exposición al no haberse concluido los estudios requeridos para su registro. Debido a su notable abundancia descrita en numerosos trabajos (Comnea-Stancu et al., 2017; Ding et al., 2019; Naidoo et al., 2020), ha sido tratado como un compuesto artificial más en los análisis realizados en esta Tesis.

De presencia más minoritaria pero igualmente importante, la polietilenimina celulosa es un polímero originado de la modificación de celulosa con polietileniminas. Éstas, de acrónimo PEI, son el segundo polímero más abundante por detrás del rayón (excluyendo los compuestos naturales). Actualmente y según señalan Riva y col. (2021), el PEI es utilizado en la remediación marina ya que adsorbe metales pesados y otros contaminantes orgánicos del medio circundante. Esta propiedad puede ser entendida desde un punto de vista positivo o negativo: el PEI sirve como compuesto "limpiador" de aguas contaminadas; pero, por el contrario, su persistencia en el medio e ingesta por parte de organismos acuáticos puede suponer la entrada en la cadena trófica de los contaminantes que lleve adheridos en elevadas cantidades. También es identificado como un compuesto citotóxico (Riva et al., 2021) y está reconocido por la ECHA como tóxico si es inhalado, y dañino para la vida acuática. Esto entraña un problema serio, ya que este compuesto se ha encontrado en todos los tipos de muestras y localizaciones estudiados en esta Tesis.

De la misma manera y presentes en todos los estudios desarrollados aquí, aunque no en todas las muestras, se encuentran otros tres polímeros con menor frecuencia. Estos son el PET (polietileno tereftalato), el cual, como el rayón, tiene el carácter de pre-registrado en la ECHA; el PAN (poliacrilonitrilo), cuya toxicidad afecta a la vida marina y produce irritación en tejidos expuestos; y el poliéster, ampliamente utilizado en prendas textiles que resulta dañino para la vida marina (https://echa.europa.eu/es/home). Finalmente, han sido identificados algunos otros compuestos en varios de los estudios que se presentan aquí. Estos son: el alquido de uretano (urethane alkid), empleado en esmalte para barcos, sin peligros recogidos para la salud en la ECHA; el PEG (glicol de polietileno), otro derivado del polietileno que resulta irritante por contacto; y el 2-etoxietil metacrilato, el cual afecta a la fertilidad y produce irritación.

Los estudios previos realizados en la costa asturiana mostraron resultados similares en lo referente a los polímeros más abundantes, con ligeras variaciones. En merluza europea, Cabanilles y col. (2022) identificaron además tres compuestos no encontrados en esta Tesis: copolímero de acetato de etilen-vinilo, pre-registrado; óxido de polialquileno, irritante y dañino para la vida marina; y tetrahidroftalamida, irritante y peligrosa por inhalación. A su vez, analizando anémonas y bígaros en la costa asturiana, Janssens y García-Vázquez (2022) añadieron a la lista el nylon y el polyvinil butiral, ambos sin daños conocidos. Por último, Masiá et al. (2019, 2020 y 2021) encontraron también policloruro de vinilo (PVC), poliestireno (PS) y metilsulfonil anilina clorhidrato, respectivamente.

A la vista de estos resultados, parece claro que la investigación sobre los efectos de los diferentes polímeros que componen los MPs marinos y las sustancias asociadas a ellos debe ser una prioridad.

2. Las algas rojas de interés gelificante como "Las grandes olvidadas"

El mercado mundial de alga roja tiene gran importancia desde hace muchos años motivada, principalmente, por el elevado valor económico y nutricional demostrado de estas algas (Kumar et al., 2008). Este caso de estudio se enmarca en el ámbito de las algas rojas de interés gelificante, las cuales producen en su pared celular y como elemento estructural agar o carragenina (Riuox & Turgeon, 2015).

Según informa la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), "*anualmente se extraen 55.000 toneladas de algas marinas con las que se producen 7.500 toneladas de agar por valor de 132 millones de dólares estadounidenses*". En el mismo reporte, indican que "*el consumo total de materias primas (algas productoras de carragenina) asciende a unas 150.000 toneladas de algas marinas, de las que se obtienen 28.000 toneladas de carragenina por un valor de 270 millones de dólares estadounidenses*" (https://www.fao.org/3/y3550s/y3550s04.htm#3.4%20Las%20algas%20rojas%20como%20fuente%20de%20agar). Estos datos ponen de manifiesto la notable importancia a nivel mundial de la industria productora de agentes gelificantes de origen vegetal.

Estos polisacáridos han surgido como sustitutos de la gelatina animal de pescado o de cerdo, al tratarse de compuestos que tienen las mismas propiedades gelificantes, pero son compatibles con el respeto a las creencias religiosas (productos kosher, halal…) así como con las elecciones alimentarias veganas y vegetarianas. Teniendo en cuenta su importancia en numerosos ámbitos (alimentación, producción de cosméticos, utilización en investigación como fungibles de laboratorio…), y tal como se muestra en los resultados de esta investigación, existe un vacío claro de información en lo referente a la presencia, acumulación y llegada al consumidor de MPs en estas algas.

En esta publicación se ponen de manifiesto cuatro puntos clave en el estudio e identificación del ciclo potencial de MPs en productos obtenidos a partir de estas algas: adsorción de MPs distribuidos en la columna de agua sobre las algas, amplificación de la cantidad de MPs durante el proceso de recogida y manejo, potencial adquisición durante el procesamiento industrial, y mala retención de MPs en las plantas de tratamiento de aguas. Hasta la fecha, sólo dos de estos cuatro puntos se encuentran entre los objetivos de los investigadores de manera activa: adsorción y plantas de tratamiento de aguas.

Respecto a la adsorción de MPs sobre las algas en el Cantábrico, la primera publicación sobre la abundancia de MPs acumulados en la superficie de algas del género *Gelidium* en esta región (Bilbao-Kareaga et al., 2023), muestra en la especie *Gelidium corneum* una concentración de 2.35 (SD 1.85) MPs/g de tejido seco. Esta cantidad es mayor que las encontradas en China por Li y col. (2022): 1.07 (SD 0.69) para la agarófita *Gracilaria lamaneiformis* y 1.95 (SD 1.49) para la carragenófita *Chondrus ocellatus*. En otros tipos de algas no gelificantes, *Pyropia spp*., el alga nori, en Japón contiene de media 1.8 (SD 0.7) MP/g (Li et al., 2020), mientras que, en el otro extremo, el alga *Endocladia muricata*, abundante en las costas de California, acumula de media 8.65 (SD 6.44) MPs/g (Saley et al., 2019). El Cantábrico sería entonces un punto intermedio respecto a la contaminación de algas por MPs, lo que hace relevante continuar con estas investigaciones dado que no todas las algas explotadas comercialmente están contaminadas por igual.

Como queda reflejado en este capítulo, existen dos puntos clave en el proceso de producción de agentes gelificantes cuya investigación no ha sido desarrollada suficientemente: el proceso de producción y recogida de algas y la manipulación industrial. En el primero de los casos, el empleo de utensilios fabricados a base de plásticos (vadeadores, guantes, recipientes de transporte…) en los procesos de producción (pesquerías) y recogida de las algas (selección, almacenamiento y transporte), podría conllevar un aumento accidental de la cantidad de MPs (Montarsolo et al., 2018). En el segundo caso, hay tres factores a tener en cuenta: el uso de utensilios de plástico en la manipulación y empaquetado (Wagner, 2017), la precipitación de MPs aéreos (8 2021), y la posible adición intencionada de MPs, tal y como sucede en los cosméticos (Duis & Coors, 2016; Anagnosti et al., 2021). La escasez de publicaciones dificulta la identificación de focos industriales y preindustriales de MPs, lo que impide la implementación de medidas que reduzcan o, al menos, limiten esta contaminación. Actualmente, el camino a recorrer para identificar todas las posibles fuentes de contaminación por MPs en el ciclo de producción y gestión de agentes gelificantes es largo. Por ello, en este capítulo, se sugieren vías de estudio nuevas o poco exploradas. Este desarrollo investigador contribuirá a mejorar el conocimiento y la calidad de los productos que consumimos de manera habitual.

Finalmente, dentro de los puntos estudiados del ciclo de producción de agentes gelificantes, las EDAR aparecen en una posición privilegiada al final del ciclo. Las investigaciones orientadas a mejorar los sistemas de retención de MPs de estas plantas de tratamiento se están reforzando tras su identificación como concentradoras de estos contaminantes (Turan et al., 2021; Egea-Corbacho et al., 2023). Los esfuerzos continuos por mejorar estos sistemas incluyen buscar nuevas alternativas. La mejora de los procesos de retención (Egea-Corbacho et al., 2023), podría conseguirse por métodos de biorremediación (Mangunwardoyo et al., 2013; Masiá et al., 2020), que permitirían la retención del 100% de los MPs que llegan a las plantas de tratamiento.

3. Los MPs en animales

3.1. Distintos biomas, la misma especie: la anguila europea.

En la presente Tesis, se ha estudiado una nueva especie animal de la costa asturiana en su relación con los MPs como contaminante, añadiéndola así a una reducida lista de organismos analizados afectados por estos compuestos en dicha región. Estos son la merluza europea (Cabanilles et al., 2022), anémonas y bígaros (Jansens & García-Vázquez, 2022), aves migratorias (Masiá et al., 2019), y mejillones (Masiá et al., 2022).

La presencia de MPs tanto en peces de medio marino como de agua dulce, se encuentra ampliamente documentada a nivel global (Pinheiro et al., 2017; Bessa et al., 2018; Digka et al., 2018; Karbalaei et al., 2019; Kasamesiri, 2020). Pero pocas investigaciones se han centrado en las especies que habitan ambos medios alternativamente (Collicutt et al., 2019; Siddiquie et al., 2022).

La anguila europea (*Anguilla anguilla*) es un caso de estudio singular, debido a su naturaleza catádroma (nacimiento en mares y reproducción en ríos) así como a su estatus de peligro crítico de extinción (Freyhof & Kottelat, 2010). La anguila europea se reproduce en el mar de los Sargazos, en el Atlántico occidental, y tras un viaje de miles de kilómetros y de hasta 2 años y medio, coloniza los ríos europeos, donde crece hasta alcanzar su madurez sexual (van Ginneken & Maes, 2005). Hasta el momento de iniciarse la presente Tesis, sólo se había publicado un caso que puso de manifiesto la presencia de MPs en esta especie (Steer et al., 2017), pero con un solo individuo analizado.

Esta Tesis expone el primer estudio en profundidad enfocado a la presencia de MPs en la anguila europea (*Anguilla anguilla*) en su etapa larvaria de "anguila de cristal", o angula. Se demuestra por primera vez la existencia de un transporte de MPs con una direccionalidad mar → río en esta especie catádroma. Durante su travesía transatlántica, las larvas leptocéfalas de anguila europea se ven expuestas a multitud de MPs, los cuales pueden adherirse a su superficie. Esta exposición es continuada (aunque con distinta intensidad) a lo largo de todo el viaje, desde el mar de los Sargazos (Carpenter & Smith, 1972), a través del Atlántico central y norte (Courtene-Jones et al., 2017; Poulain et al., 2018), y hasta su llegada a la desembocadura de los ríos europeos (Atwood et al., 2019; Scherer et al. 2020). Una vez las angulas llegan a estos ríos con un tamaño de aproximadamente 70 mm, suben aguas arriba y permanecen en ellos hasta 20 años, cuando adquieren su madurez reproductiva y vuelven a migrar al mar de los Sargazos para desovar (Deedler 1970).

Los resultados obtenidos en esta investigación muestran un máximo de MPs/g en las anguilas del río Cabra, con 2.736 (SD 0.061). Un estudio reciente en angulas del suroeste británico durante su transición mar-río (Parker et al., 2023) ha reportado una concentración media de 0.03 MPs/individuo, de forma que los resultados de esta investigación sugerirían una mayor contaminación de este recurso en las aguas cantábricas. Una explicación adicional podría encontrarse en la diferente metodología empleada en ambos estudios. Parker et al. (2023) emplearon hidróxido de potasio y filtros de 48 µm de poro, mientras que en esta Tesis se utilizó peróxido de hidrógeno y filtrado a través de poros de 0.45 µm. El diferente tamaño de poro (dos órdenes de magnitud) puede jugar un papel muy importante en el proceso de retención de partículas durante la filtración; en el primer caso se perderán partículas menores de 48 µm que son retenidas con el filtro de 0.45 µm. Por otra parte, García y col. (2021) habían reportado una ausencia de MPs en anguilas recogidas a lo largo del río Garona y sus afluentes (suroeste de Francia). Esta publicación no es realmente comparable debido, primero, a la zona de captura (no en la zona de transición mar-río sino aguas arriba), y en segundo lugar a que únicamente analizaron 11 individuos, mientras que Parker y col. (2023) estudiaron 300 ejemplares, una cifra mucho más cercana a las 380 angulas estudiadas en esta Tesis.

Asimismo, los resultados mostraron que las angulas se contaminan a su llegada a los ríos principalmente por MP de origen fluvial, tal y como se espera por el importante papel de los ríos en el aporte de MP al mar (Park et al., 2020; Heshmati et al., 2021). Secundariamente, la contaminación descrita para las angulas del río Nalón también se puede explicar a partir de los datos obtenidos del agua del mar. De esta manera se propone una contaminación dual sobre esta especie cuando los organismos se encuentran en el límite entre biomas.

Como se mencionó previamente, en este capítulo se presenta evidencia de contaminación por MPs desde el agua de mar al río, como demuestra la presencia de partículas de plástico (alquil-utetano) alojadas en el tejido muscular lateral de dos anguilas. Actualmente se encuentra bien documentada la presencia de MPs que traspasan la barrera gastrointestinal una vez ingeridos y se alojan en el músculo dorsolateral de peces (Barboza et al., 2020; Atamanalp et al., 2021; Makhdoumi et al., 2023). Sin embargo, aunque el proceso de translocación aún se encuentra en estudio y no se conoce en profundidad (Zeytin et al., 2020), en principio, se podría producir por rotura directa del tejido digestivo (Jabeen et al., 2018). En este caso particular, esta hipótesis quedaría descartada debido al tamaño de al menos una de las partículas (Figura 1.1), que en comparación con el tamaño del animal es tan grande, que el proceso no sería compatible con la vida, produciendo un daño permanente y/o letal.

Figura 1.1: Fragmento de alquil-uretano extraído del músculo dorsolateral de una angula europea. Fotografía de D. Menéndez.

La explicación alternativa sería la adhesión de estos fragmentos a la superficie del organismo en fases larvarias tempranas o incluso prelarvarias. A continuación, el músculo se desarrollaría alrededor del MP, incluyéndolo como un elemento estructural del animal. Los fragmentos de estas angulas fueron identificados químicamente como alquil-uretano, un esmalte plástico utilizado en la cubierta de barcos, lo que concuerda con una contaminación en el mar. Una vez en el río, estos MPs se podrían depositar en el fondo si la angula muere, o ser incluidos en la cadena trófica si es ingerida, afectando así a otras especies (Carbery et al., 2018; Smiroldo et al., 2019; da Costa-Araújo et al., 2020). De esta manera se presenta una nueva vía de entrada de MPs a los ríos transportados por especies diádromas cuyo potencial es aún desconocido.

3.2. El último eslabón de la cadena. La merluza negra en aguas africanas.

Como último organismo analizado en esta Tesis y para cerrar la investigación con un depredador superior de la cadena trófica, se ha estudiado la presencia, abundancia y composición de los MPs en la merluza negra o de Benguela (*M. polli*).

Esta merluza, pescada en las costas noroeste de África, forma parte de un género conformado por 12 especies (Blanco-Fernández et al., 2021), ampliamente distribuidas a nivel mundial, cuya investigación en materia de MPs se ha concentrado en la merluza europea (*Merluccius merluccius*), y en menor medida en *M. bilinearis*, *M. capensis*, *M. gayi*, *M. paradoxus* y *M. productos*. Los resultados de esta investigación en comparación con los resultados obtenidos para otras especies de merluza se recogen en la Tabla 1.1. En este trabajo, el 100% de las merluzas analizadas se describen como portadoras de MPs en al menos uno de sus tejidos analizados. También se encontraron en los tres individuos de *M. merluccius* del Cantábrico central analizados por Cabanilles y col. (2022). Sin embargo, hay casos como *M. merluccius* del Golfo de Cádiz (Bellas et al., 2016) o *M. gayi* de la costa chilena (Paredes-Osses et al., 2019) donde el porcentaje de individuos con MPs es menor del 20%. Estas diferencias entre trabajos, realizados siguiendo una misma metodología, reflejan la gran diferencia en contaminación por MPs entre zonas marinas.

En la Tabla 1.1 se aprecia que la mayoría de los estudios de MPs en merluzas se han realizado sobre el tracto gastrointestinal. Esto pasa también en otros géneros y especies como *Sparus aurata* (Sánchez-Almeida et al., 2022) o *Scomber colias* (Herrera et al., 2019). La investigación de MPs en peces se ha centrado en su ingesta, mientras que su acumulación en tejidos distintos del tracto gastrointestinal (GIT) está poco estudiada (McIlwraith et al., 2021; Ferrante et al., 2022). Centrándose en el hígado, los resultados en *M. polli* muestran una cantidad de MPs significativamente superior a otras especies de merluza estudiadas. Desde el este de Canadá en *M. bilinearis* (Liboiron et al., 2018) hasta el sur de Sudáfrica en *M. capensis* y *M. paradoxus* (Sparks & Immelman, 2020), la concentración de MPs/g es menor que la descrita aquí para *M. polli* de 0.605 (SD 0.595) MPs/g en hígado (Tabla 1.1). Por otro lado, los resultados publicados por Cabanilles y col. (2022), en merluza europea pescada en la costa cantábrica, muestran valores de un orden de magnitud (10 veces) superior con respecto a la merluza de Benguela analizada en esta Tesis. En este caso es necesario tener en cuenta la diferencia en el número de muestras analizadas, 94 en el caso de la merluza de Benguela frente a 3 merluzas europeas.

Estos datos son de gran interés ya que la vía de entrada más común de MPs al organismo de los peces es el GIT (Dantas et al., 2020; Nunes et al., 2021), llegando al hígado tras la traslocación a través de conductos, cavidades y tejidos (Yu et al., 2018). De forma más general, los datos apuntarían a una mayor contaminación de MPs en merluzas respecto a otras especies de peces. Estudios realizados en la misma área sobre los peces pelágicos *Dicentrarchus labrax* y *Sparus aurata* por Sánchez-Almeida y col. (2022), han mostrado unas concentraciones de 0.0081 (SD 0.0907) y 0.0084 (SD 0.0405) MPs/g en GIT respectivamente, lo que dista de los resultados obtenidos para el género *Merluccius.*

Una explicación plausible de este fenómeno podría ser que la merluza de Benguela, debido a su distribución demersal, tenga un mayor acceso a MPs de la columna de agua que aquellas especies localizadas a menores profundidades. Hasta la fecha, esta hipótesis no ha sido demostrada y varias investigaciones son contrarias a ella (Lusher et al., 2013; Murphy et al., 2017; Suwartiningsih et al., 2020). Otra posible explicación sería la biomagnificación resultante de la transferencia trófica de MPs, siendo la merluza de Benguela un depredador voraz e, incluso, caníbal (Kilongo & Mehl, 1998). La biomagnificación ha sido ampliamente demostrada en el medio natural en múltiples regiones y organismos (Alava, 2020; Bhatt et al., 2022; Miller et al., 2023), por lo que sería probablemente una interpretación más razonable.

Por último, la exposición a MPs en el hígado ha demostrado ejercer un efecto negativo sobre el estado fisiológico de las merluzas. Este efecto ha sido evaluado a través de metodología correlacional: cuanto mayor es la concentración de MPs/g, menor es el factor de condición del animal. Resultados publicados por otros autores apuntan a fenómenos similares en otras especies de peces como *Girella leaviformis* o *Sardina pilchardus* (Mizraji et al., 2017; Compa et al., 2018), que reflejan un deterioro del estado fisiológico del animal asociada a una acumulación acusada de MPs, con una $r = -0.234$ y $p=0.024$, en este estudio particular.

Tabla 1.1: Estudios publicados sobre MPs en especies de merluzas a nivel global.

Nombre común	*Nombre científico*	Región	Distribución	Tejido estudiado	N	Media [MP/g]	SD	Mayor [MP/g]	Menor [MP/g]	% de presencia	Referencia
Merluza de Benguela	*Merluccius polli*	Noroeste Africano	Batidemersal	Músculo	94	0.181	0.111	0.472	0	94	Menéndez et al., 2023
Merluza de Benguela	*Merluccius polli*	Noroeste Africano	Batidemersal	Hígado	94	0.605	0.595	2.976	0	83	Menéndez et al., 2023
Merluza de Benguela	*Merluccius polli*	Noroeste Africano	Batidemersal	Branquias	94	0.517	0.41	1.762	0	80	Menéndez et al., 2023
Merluza europea	*Merluccius merluccius*	Golfo de Vizcaya	Demersal	TGI	3	3.896	2.758	5.77	0.73	100	Cabanilles et al., 2022
Merluza europea	*Merluccius merluccius*	Golfo de Vizcaya	Demersal	Hígado	3	2.85	3.089	6.4	0.77	100	Cabanilles et al., 2022
Merluza europea	*Merluccius merluccius*	Golfo de Vizcaya	Demersal	Branquias	3	5.19	3.119	8.49	2.29	100	Cabanilles et al., 2022
Merluza europea	*Merluccius merluccius*	Costa portuguesa	Demersal	TGI	7	0.29*	0.49*				Neves et al., 2015
Merluza europea	*Merluccius merluccius*	Mercado local	Demersal	TGI	5	0.40*	0.89*				Neves et al., 2015
Merluza europea	*Merluccius merluccius*	Norte del mar Tirreno	Demersal	TGI completo	36	0.013					Giani et al., 2019
Merluza europea	*Merluccius merluccius*	Mar Adriático	Demersal	TGI completo	36	0.065					Giani et al., 2019
Merluza europea	*Merluccius merluccius*	Mar Jónico	Demersal	TGI completo	25	0.16					Giani et al., 2019
Merluza europea	*Merluccius merluccius*	Costa Tirrena de Sicilia	Demersal	Contenido del TGI	67	0.1944		0.125 (Machos)	0.087 (Hembras)		Mancuso et al., 2019

Merluza europea	*Merluccius merluccius*	Mar Jónico	Demersal	TGI	36	0	0	0	0		Anastasopoulou et al., 2013
Merluza europea	*Merluccius merluccius*	Costa de Montenegro	Demersal	TGI	50	0.007	0.008				Boskovic' et al., 2022
Merluza europea	*Merluccius merluccius*	Mar Adriático norte	Demersal	TGI	30	0.011	0.012				Mistri et al., 2022
Merluza europea	*Merluccius merluccius*	Golfo de Cádiz	Demersal	TGI						16.7	Bellas et al., 2016
Merluza del Pacífico	*Merluccius productus*	Ecosistema de la bahía de Monterrey	Pelágica	TGI	5	1.2*	1.6*	4*	0		Hamilton et al., 2021
Merluza de aguas superficiales del Cabo	*Merluccius capensis*	Banco de las agujas, Sudáfrica	Demersal	TGI	15	0.25	0.03				Sparks & Immelman, 2020
Merluza de agua profunda del Cabo	*Merluccius paradoxus*	Banco de las agujas, Sudáfrica	Demersal	TGI	15	0.15	0.04				Sparks & Immelman, 2020
Merluza plateada	*Merluccius bilinearis*	Este de Canada	Bentopelágica	Contenido del TGI	134	0	0	0	0		Liboiron et al., 2018
Merluza del Pacífico sur	*Merluccius gayi*	Chile Central	Batidemersal	Contenido del TGI	10					10	Paredes-Osses et al., 2019

+ Las concentraciones marcadas con asterisco (*) indican cálculos de MP/individuo, no por gramo de tejido

++ TGI=Tracto Gastrointestinal

Espacio en blanco: Dato no disponible

Finalmente, este es uno de los primeros estudios realizados en peces adultos acerca de la influencia que ejercen los MPs sobre la integridad del material genético. Pennetier y col. (2020) demostraron un efecto tóxico y subletal de los MPs sobre larvas de peces de la especie *Oryzias latipes,* conocido comúnmente como Meraka o pez arroz japonés. En su trabajo emplearon el ensayo del cometa para demostrar la alteración del ADN. Sus resultados no son directamente comparables con los presentados en esta Tesis. El ensayo empleado aquí para determinar la degradación del ADN en el hígado de merluza fue la visualización directa en gel de agarosa, pero se puede concluir que los MPs se asocian con una degradación significativa en ambos casos. En este caso de estudio, existe una correlación significativa positiva, es decir, a mayor número de MPs, mayor degradación sufre el ADN ($r = 0.35$, $p= 0.015$). El proceso de degradación del ADN podría responder a un efecto físico de los MPs, actuando mecánicamente como "destructores" de tejidos (Li et al., 2018). El ensayo del cometa de Pennetier y col. (2020) se hizo sobre peces que estaban vivos durante el experimento, mientras que la degradación extrema observada en muchos individuos en esta Tesis implicaría un hígado totalmente destruido incompatible con la vida. Este efecto físico puede venir determinado y potenciado por el proceso de pesca de arrastre. Este somete a las merluzas a un cambio repentino de presiones (Suuronen, 2005), lo que genera descompresiones repentinas y daña el tejido de los órganos internos. Los MPs durante esa descompresión actuarían contribuyendo a desgarrar el tejido y romper las células. En el experimento de Pennetier y col. (2020) podría también haber un efecto citotóxico (Hahladakis et al., 2018; Wang et al., 2022), algo que parece improbable como causa principal de rotura de ADN propuesto aquí para las merluzas, probablemente relacionado con el proceso rápido de descompresión, debido al cambio brusco de presiones en el organismo.

4. Recomendaciones para la eliminación de MPs y mitigación de sus impactos ambientales.

En las publicaciones derivadas de la presente Tesis surgen varias propuestas cuya finalidad es limitar el acceso al mar de MPs, así como la posible mitigación de sus efectos en los organismos afectados y en el consumidor humano. De dichas propuestas surgen tres posibles medidas. En primer lugar, una es de carácter técnico: la mejora de los sistemas de prevención, que limitan la entrada de contaminantes al medio marino (EDAR), ha de continuar ganando importancia (Ziajahormi et al., 2017; Bayo et al., 2020). Dentro de este punto, se ha de poner especial énfasis en los residuos primarios producidos en estas plantas de tratamiento. Estos residuos llamados biosólidos son utilizados habitualmente como fertilizantes (Corradini et al., 2019; Rolsky et al., 2020), pero contienen elevadas cantidades de MPs que penetran en el suelo, llegando a aguas subterráneas y, de nuevo a plantas de tratamiento de aguas o directamente al mar (Freeman et al., 2020). La utilización de microorganismos en los procesos de biodegradación de plástico podría aumentar la eficacia de retención de MPs de estos sistemas hasta un valor cercano al 100% (Masiá et al., 2020; Tang & Hadibarata, 2022), y aunque cuando se publicaron estos artículos se trataba de una propuesta teórica, aún no demostrada en la práctica, actualmente hay nuevas perspectivas. Otros organismos de mayor tamaño, recogidos en Kirshnan y col. (2023) y propuestos para bioremediación son: plantas marinas como el alga *Fucus* o la macrófita *Thalassia*, las cuales secuestrarían los MPs del agua a través de sus organismos epibiontes así como mediante su superficie mucilaginosa, gusanos marinos de la especie *Arenicola marina* por ingesta o adhesión y mejillones de la especie *Mytilus edulis* (mejillón azul) por adhesión y acumulación. En el año 2019, Mohsen y col. reportaron la acumulación de MPs en el líquido celómico de pepinos de mar de la especie *Apostichopus japonicus.* Estos organismos detritívoros podrían ser también utilizados como biorremediadores, orientados principalmente a la eliminación de MPs en el sedimento, el cual ingieren y del cual toman nutrientes. Finalmente, varios grupos han identificado los *biofilms* (población de células que crecen juntas rodeadas de una matriz de polisacáridos – Lewis, 2001) como potenciales y efectivos degradadores de MPs, proceso mediado a través de las enzimas catalíticas implicadas en su metabolismo (Abomonye et al., 2021; Debroy et al., 2021; Sun et al., 2023).

Alternativamente, se pueden apuntar dos medidas de carácter social. La medida quizás más importante es la educación medioambiental, proporcionando una información veraz y asequible para todos los grupos de la población, independientemente de su conocimiento previo o de sus estudios. La eliminación o, al menos, una reducción de la producción de desechos plásticos puede reducir notablemente el problema ya que se limitaría la generación de MPs. Promover el reciclaje, la reducción o supresión del consumo de plástico, junto con las conductas cívicas respecto a la basura, es una prioridad. Actualmente, el conocimiento poblacional en materia de contaminación por MPs es escaso e irregular, aunque se encuentra en constante crecimiento. Este conocimiento parece ser mayor en Europa con respecto al resto del mundo (García-Vázquez & García-Ael, 2021), menor en clases medias y bajas desde un punto de vista socioeconómico (Catarino et al., 2021), y mayor en mujeres que en hombres (Dowarah et al., 2022). Las redes sociales e internet funcionan activamente como los principales canales de conocimiento y acercamiento a los MPs por parte de la población (García-Vázquez & García-Ael, 2021; Yu & Ma, 2022), por lo que juegan un papel capital en la educación medioambiental de la población. Pero el peso de una educación efectiva, de calidad y abierta a todos los públicos no puede recaer exclusivamente en las redes sociales. Actualmente, aparte de las redes sociales, se han planteado diversas actividades para un público general a fin de concienciar a la población a cerca del cambio global y de la importancia de la investigación, limitación de uso y promoviendo un reciclaje correcto de los MPs. Algunas medidas propuestas son: Campañas de concienciación o pago directo por el reciclaje (Deme et al., 2022), competiciones de conocimiento (Yu & Ma, 2022), comunicación científica accesible y de calidad (García-Vázquez & García-Ael, 2021) e incluso la formación dirigida a grupos específicos como los pescadores (Munhoz et al., 2023). Aún queda un largo camino por recorrer, pero la creciente concienciación y percepción acerca de estos contaminantes muestra una dirección positiva para un futuro sostenible.

En último lugar, y pese a las numerosas propuestas y políticas aplicadas para detener la creciente contaminación por plásticos (Munhoz et al., 2023), se hace necesaria la implementación de leyes ambientales efectivas a corto plazo y de ámbito global. Estas deberán ser destinadas a la reducción de desechos tanto urbanos, incluidos los hogares, como industriales. Asimismo, una legislación más exigente en lo relativo al delpósito de residuos en ríos, lagos y playas (Narloch et al., 2022), incluyendo las actividades pesqueras como fuente activa de contaminación (Montarsolo et al., 2018). Hasta la fecha, se ha observado cómo la aplicación de medidas para reducir el consumo de plástico es efectiva para concienciar a la población y promover un consumo responsable de estos materiales, lo que reduce enormemente su potencial llegada al medio ambiente. Un ejemplo claro es el de México. Desde 2020, México ha ido aumentando sus limitaciones al uso innecesario de plástico, específicamente en la utilización de plásticos de un solo uso mientras que, en España, esas limitaciones son, aún, insuficientes (García-Vázquez et al., 2022). Leyes orientadas a la eliminación de MPs primarios (microesferas) en cosméticos, así como para reducir el consumo de bolsas de plástico de un solo uso se están llevando a cabo actualmente en multitud de países (Xanthos & Walker, 2017). Lamentablemente, su incumplimiento en países en desarrollo, así como la falta de una legislación global no contribuye a una reducción efectiva en la liberación de estos MPs al mar (Onyena et al., 2022).

Estas acciones, adoptadas individualmente contribuyen de manera considerable a solucionar la problemática derivada de la presencia de MPs en el mar. Por otro lado, su aplicación conjunta (desde una perspectiva global) debería, si no evitar totalmente la liberación de MPs al medio marino, al menos limitarla de manera notable de forma que este compartimento de la biosfera no se vea afectado significativamente por la actividad antrópica en futuras generaciones.

Como propuestas más específicas y relativamente sencillas por su inmediatez, se sugiere la limpieza de ríos utilizados por la anguila europea para crecer, y la de playas próximas a las algas explotadas para producción de agar. Eliminar los plásticos prevendría, al menos parcialmente, la acumulación de microplásticos secundarios en la desembocadura. De esta manera se reduciría el efecto antrópico producido sobre especies como la anguila, en peligro crítico de extinción. La implicación de voluntariado en estas acciones cumpliría además una doble función: abaratar el coste de las limpiezas y aumentar la educación y concienciación pública respecto al problema de los plásticos y microplásticos, que es muy superior en las personas, tanto adultos como niños, que participan en este tipo de voluntariado medioambiental (Rayon-Viña et al., 2019).

Conclusiones

I. Se ha evidenciado una ausencia notable de conocimiento sobre la acumulación de MPs en el proceso de cultivo, recolección y procesamiento industrial de algas marinas gelificantes. Según la investigación llevada a cabo en esta Tesis, los puntos clave en el ciclo de producción de agentes gelificantes cuya investigación sería necesaria para conocer el estado actual y aplicar medidas de mitigación son: depósito y adhesión de MPs en la superficie de las algas de interés gelificante, adición inintencionada o accidental de MPs durante el proceso de manipulación de las algas, así como durante el procesado industrial (microplásticos aéreos u originados durante el empaquetado), y mejora de los sistemas de retención de MPs en las plantas de tratamiento de aguas residuales.

II. Se ha encontrado una elevada cantidad de MPs en la anguila europea (*A. anguilla*), una especie en peligro de extinción crítico, a su entrada en tres ríos asturianos. Se demuestra por primera vez que esta especie transporta MPs desde el mar hasta el río. La anguila europea bioacumula MPs, llegando a concentraciones en tejido varios órdenes de magnitud superiores a los del medio circundante.

III. La merluza de Benguela (*M. polli*) acumula MPs en el hígado y las branquias, mientras que el músculo, como tejido comestible, presenta los valores más bajos. La concentración de MPs en este órgano afecta de manera negativa al estado fisiológico del animal, empeorando su factor de condición y el nivel de degradación del ADN, probablemente durante la extracción pesquera. Es posible que los MPs afecten de forma especial a las especies demersales, un aspecto que debería ser investigado.

IV. Se recomienda proceder a una depuración efectiva de las aguas asturianas, incluyendo mejora de la retención de MPs en las plantas de tratamiento de aguas mediante biorremediación, y limpiezas organizadas de ríos y playas, así como al control medioambiental que limite la llegada de MPs a esta costa. La adquisición de MPs en medio marino por la anguila europea acentúa la necesidad de medidas políticas a nivel global que pongan remedio al problema de los MPs en el medio marino y de agua dulce. Otra propuesta desde esta Tesis apoyada por la investigación social es la educación general, para el consumo responsable y la educación medioambiental, para reducir la demanda de plástico y la producción de desechos.

Conclusions

I. There has been a notable lack of knowledge about the accumulation of MPs in the process of cultivation, harvesting and industrial processing of marine gelling algae. According to the research carried out in this Thesis, the key points in the production cycle of gelling agents whose investigation would be necessary to know the current state and apply mitigation measures are: deposit and adhesion of MPs on the surface of the algae of interest gelling agent, unintentional or accidental addition of MPs during the algae handling process, as well as during industrial processing (aerial microplastics or originated during packaging), and improvement of MPs retention systems in WasteWater Treatment Plants .

II. A high amount of MPs has been found in the European eel (*A. anguilla*), a critically endangered species, upon entering three Asturian rivers. It is shown for the first time that this species transports MPs from the sea to the river.

III. The Benguela hake (*M. polli*) accumulate MPs in the liver and gills, while muscle, as edible tissue, presents the lowest values. The concentration of MPs in this organ negatively affects the physiological state of the animal, worsening its condition factor and the level of DNA degradation, probably during fishing extraction. It is possible that MPs affect demersal species in a special way, an aspect that should be investigated.

IV. It is recommended to proceed with an effective depuration of Asturian waters, including improvement of the retention of MPs in WasteWater Treatment Plants through bioremediation, and organized cleaning of rivers and beaches, as well as environmental control that limits the arrival of MPs to this coast. The acquisition of marine MPs by the European eel accentuates the need for global policy measures to remedy the problem of MPs in the marine and freshwater environment. Another proposal from this Thesis supported by social research is general education for a responsible consumption and environmental education to reduce the demand for plastic and the production of waste.

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Materiales suplementarios

Supplementary table 1. Morphological characteristics of the eel samples analysed. Mean length and weight, and proportion of individuals in pigmentation stages. Briefly, VA: pigmentation is located at the end of the caudal region and does not affect the rest of the body, VB: pigmentation exceeds the caudal end and does not progress along the body; VIA0: dorsal surface pigmentation: - at least one point behind the brain line, but not a continuous border; dorso- and medio-lateral pigmentation: VIA1, VIA2; pre-anal pigmentation: VIA3 (Elie et al., 1982).

	Nalón	**Bedón**	**Cabra**
Mean length	74.9 (3.9)	74.04 (4.01)	71.48 (5.53)
Mean weight	0.36 (0.06)	0.37 (0.07)	0.35 (0.09)
Stage			
VA	0.06	0.08	0
VB	0.94	0.86	0.4
VIA0	0	0.06	0.2
VIA1	0	0	0.18
VIA2	0	0	0.12
VIA3	0	0	0.1

Supplementary table 2. Number of MPs of different types and colours found in this study. E = glass eels, W = river water, S = sediments.

	Fibres							Fragments				
	Black	Blue	Dark/ Grey	Transparent/ White	Reddish	Green	Total	Blue	Black	Green	Others	Total
E-Nalón	23	19	4	3	3	1	53	0	0	0	0	0
E-Bedón	41	48	0	2	1	2	94	7	1	0	0	8
E-Cabra	30	19	5	0	4	0	58	4	0	0	0	4
W-Nalón	29	21	0	4	3	0	57	1	0	0	2	3
W-Bedón	46	35	3	15	2	0	101	2	0	2	2	6
W-Cabra	261	56	5	11	9	4	346	4	0	0	1	5
S-Nalón	17	5	0	11	0	1	34	1	0	0	0	1
S-Bedón	29	15	1	10	0	2	57	2	0	0	0	2
S-Cabra	21	19	5	4	1	1	51	0	0	0	0	0
Seawater	7	4	6	7	5	6	35	1	5	5	0	11

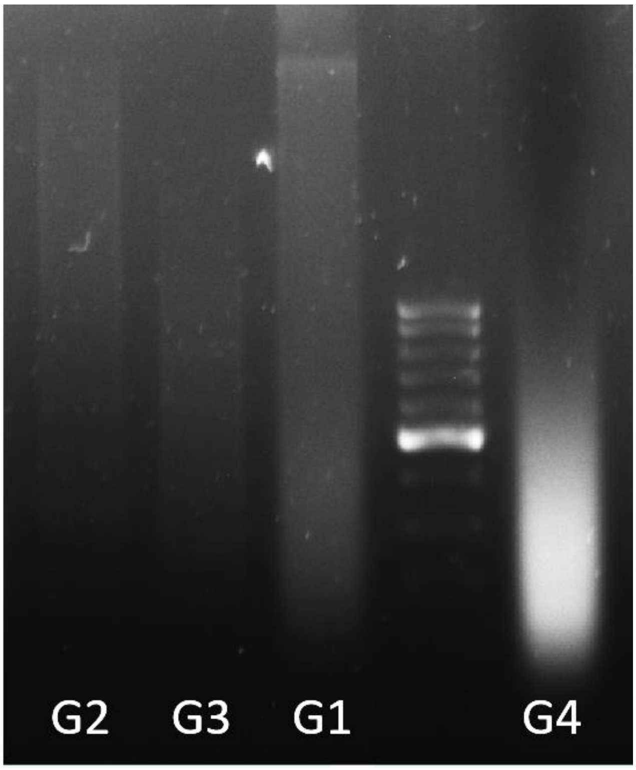
Supplementary table 3. Multiple regression analysis with the profiles (by shape and colour) of MPs in glass eels and in environmental components as dependent and independent variables respectively. Significant values are marked in bold.

Dependent variable: Nalón eel profiles	Coefficient	SE	t	p-value	R^2
Constant	-1.65	1.0	1.65	0.15	
Nalón water	**0.91**	**0.07**	**12.78**	**0.00004**	**0.96**
Nalón sediments	-0.30	0.14	2.19	0.07	0.55
Seawater	**0.55**	**0.22**	**2.52**	**0.04**	**0.11**
Dependent variable: Bedón eel profiles					
Constant	2.54	4.69	0.55	0.61	
Bedón water	**1.95**	**0.56**	**3.47**	**0.01**	**0.86**
Bedón sediments	-1.56	0.99	1.56	0.18	0.73
Seawater	-0.88	0.98	0.90	0.40	0.02
Dependent variable: Cabra eel profiles					
Constant	2.46	1.55	1.59	0.16	
Cabra water	**0.05**	**0.02**	**3.53**	**0.01**	**0.84**
Cabra sediments	**0.80**	**0.15**	**5.22**	**0.002**	**0.91**
Seawater	-0.49	0.32	1.54	0.17	0.06

Supplementary table 4. Number of items of different artificial materials identified from Fourier-Transformed Infrared spectroscopy in this study. UA, urethane alkyd; PEI, Polyethyleneimine; PE, polyethylene; PEG, polyethylene glycol; PAN/PAA, acrylic or poly-acrylic; PET, polyethylene terephthalate; PP, polypropylene. E, W and S are for glass eels, river water and river sediments, respectively.

	Rayon	UA	PEI	PET	PAN/PAA	PP	Polyester	PEG	PE	Total
E-NALON	6	0	4	0	1	0	0	1	0	12
W-NALON	2	0	3	0	0	0	1	1	0	7
S-NALON	9	0	0	1	1	0	0	0	0	11
E-BEDON	6	0	3	0	0	1	0	0	0	10
W-BEDON	4	0	3	0	0	1	0	1	0	9
S-BEDON	7	0	3	2	0	0	1	0	0	13
E-CABRA	5	2	0	3	0	1	0	0	1	12
W-CABRA	7	0	5	1	1	0	0	2	0	16
S-CABRA	9	0	0	0	0	0	1	0	0	10
SEAWATER	6	0	2	1	4	2	6	0	9	30

Supplementary Figure 1: Different degradation levels of genomic DNA according to Masiá et al. (2021), with a clear band of large-size DNA (G1), fragments relatively long (G2), fragments of medium size (G3) and small fragments (G4). The ladder (100-1000 bp) is between G1 and G4



Supplementary Table 1. Tissue digested (g) and particle counts (MPs) in the *Merluccius polli* individuals analysed in this study.

Sample code	Total Weight (g)	Total length (cm)	Gill digested (g)	Gill MPs	Liver digested (g)	Liver MPs	Muscle digested (g)	Muscle MPs
1	402.94	37.8	2.96	2	4.82	2	19.7	5
2	362.05	36.1	2.85	2	4.06	1	16.43	0

3	382.46	34.7	3.15	2	5.9	2	22.28	6
4	279.8	31.2	2.37	2	1.97	1	16	1
5	341.66	34.7	3.12	3	5.2	0	22.94	3
6	607.5	40.1	5.41	5	5.95	3	24.27	4
7	404.24	36.2	2.82	2	3.29	2	20.32	7
8	510.02	39.5	5.35	3	4.7	10	24.55	9
9	542.7	40.8	5.77	3	5.7	3	20.21	8
10	526.3	38.3	4.6	5	4.5	2	26.4	4
11	391.75	36.1	3.61	5	5.3	2	16.95	8
12	483.63	36.7	3.68	0	4.9	0	16.62	2
13	480.57	38.2	3.9	3	5.6	2	22.51	2
14	594.5	40.4	2.53	2	4.79	4	25.3	5
15	359.9	32.6	3.21	1	3.72	3	18.86	6
16	659.6	42.7	5.44	0	5.2	1	20.18	5
17	490.2	37.1	3.8	5	5.13	3	19.59	7
19	366.7	35.5	3.1	2	3.6	1	13.63	1
21	305.6	42.4	5.06	0	5.04	15	20.01	2
22	487.26	37.6	4.38	2	4.43	2	18.92	2
23	583.88	41.2	4.96	4	5	4	27.5	4
24	399.15	36	4.63	0	4.73	2	16.6	3
25	504.86	41.4	3.99	1	4.77	1	19.9	3
26	524.3	39.4	4.76	3	5.59	0	21.16	2
27	347.96	36.9	3.25	1	5.1	1	20.15	1
28	612.16	39.8	5.2	1	3.2	3	21.39	1
29	468.6	37.2	3.94	3	1.88	1	17.49	5
30	493.4	38.6	4.2	1	3.59	0	24.95	3
31	606.56	41.8	4.74	0	5.3	8	20.1	2
32	568.28	40.1	4.74	2	5.82	1	26.22	8
33	483.8	38.4	3.26	3	5.84	4	14.31	3
34	616.2	39.8	3.56	2	6.2	1	16.55	4
35	441.4	37	4.29	0	5.25	2	19.75	9
36	571.13	37.8	3.63	1	5	4	28.56	3

37	433.3	35.3	3	3	5	0	13	1
38	696.44	42.8	5.11	0	5	4	28.2	6
39	347.96	34	3.14	0	6.31	1	15.5	2
40	564.95	38.1	5.38	0	5.91	3	21.05	0
41	522.57	38.8	3.52	2	4.28	1	14.44	0
42	528.13	38.6	4.29	1	6.6	7	21.08	5
43	604.8	40.3	5.52	1	4.54	5	20.83	2
44	516.4	39.7	4.2	1	4.91	0	20.51	5
45	341.2	33.4	3	1	5	2	20	1
46	360.67	34.5	3.07	3	2	2	20.9	5
47	414.19	36.1	3.07	1	4.58	5	13.65	1
48	578.9	39.5	4.16	0	Not available		14.49	2
49	360.9	34.5	3.2	0	2.6	0	14.9	3
50	373.17	34.5	3.13	3	4.99	2	28.54	3
51	368.11	34.1	2.72	0	3.97	2	27.4	7
52	454.1	36.2	3.12	1	6.19	5	20.68	5
54	509.28	40.3	4	2	5.6	1	26.4	4
55	526.4	41.7	4.5	3	5.1	1	20.03	5
56	543.1	40.9	5.1	4	1.76	1	26.55	12
57	570.8	39.4	4.94	1	4.66	2	17.59	1
58	341.65	34	3.49	2	4.7	3	19.79	2
59	371.36	33	3.74	2	2.38	0	16	3
60	355.36	34.2	3.23	1	5.9	1	22.78	3
61	516.6	38.7	3.84	2	5.03	3	20.6	3
62	528.62	39.5	3.73	3	5.9	1	26.79	6
63	411.5	34.2	3	2	5.5	2	20	3
64	479.2	37.5	3.15	3	5.03	1	19.95	2
65	372.3	34.9	3.33	3	5.3	0	25.22	8
66	492	38.3	4.07	1	4.6	11	23.1	3
67	498.07	38.3	3.99	0	4.06	5	30	8
68	305.28	33	2.86	0	2.72	1	15.44	1

69	403.01	36.2	4.96	4	4.96	3	21.5	7
71	446.81	38.5	3.6	4	3.75	0	14.66	4
72	314.39	32.4	3.47	1	1.94	3	12.05	0
73	503.39	39	3.45	5	6.6	3	23.94	4
74	449.83	36.9	3.6	1	2.12	3	24.2	8
75	318.68	33.2	3.29	4	3.22	1	17.88	3
76	425.68	34.25	4.32	2	2.92	2	20.83	6
77	605.25	38.4	6.61	1	5.02	3	14.12	3
78	376.56	34	3.64	1	4.1	0	20.62	3
79	401.16	36.5	2.63	1	1.02	1	20.19	4
80	441.3	36.5	3.3	1	2.85	0	22.01	2
81	368.34	35.2	2.75	3	4.23	4	16.49	5
82	419.18	36.5	3.84	1	3.63	1	20.12	4
83	419.33	35.4	3.33	0	4.86	0	31.6	7
84	281.38	31	3.94	3	4.4	2	13.49	3
85	382.66	35.2	4.3	1	2.1	2	15.25	5
86	335.14	32.86	2.27	4	1.17	3	9.65	2
88	470.18	36.4	4.49	2	5.23	0	24.22	4
89	382.41	35.1	3.8	4	4.6	1	12.7	6
90	333.47	33.3	3.3	1	5.27	1	15.45	2
92	368.87	36.6	2.5	2	2.1	1	20.37	0
93	434.8	37.5	3.85	0	2.01	4	18.43	3
94	408.77	37.5	3.75	0	2.75	4	17.8	3
95	457.65	34.5	3.62	2	5.16	0	17.69	0
96	381.4	35	2.5	3	2.8	4	16	2
97	359.73	34.1	2.66	0	2.94	3	22.55	2
98	430.65	36	3.69	4	4.8	7	25.08	1
99	419.58	34.2	3.79	0	5.29	3	18.87	3
100	362.01	35	3.28	3	2.7	2	19.08	2

Supplementary Table 2. Condition factor and DNA degradation degree found in liver ofthe individuals analysed in this study.

Sample code	MP/g	Fulton's *k*	DNA Degradation Degree (Masiá et al., 2021)
21	2.97619048	0.401	4
86	2.56410256	0.945	3
66	2.39130435	0.876	4
8	2.12765957	0.828	3
72	1.54639175	0.924	3
31	1.50943396	0.831	4
94	1.45454545	0.775	1
74	1.41509434	0.895	3
67	1.23152709	0.887	2
47	1.09170306	0.880	3
42	1.06060606	0.918	3
97	1.02040816	0.907	3
46	1	0.878	1
85	0.95238095	0.877	3
81	0.94562648	0.845	3
28	0.9375	0.971	4
14	0.83507307	0.902	2
52	0.80775444	0.957	3
38	0.8	0.888	3
36	0.8	1.057	2
100	0.74074074	0.844	2
33	0.68493151	0.854	3
76	0.68493151	1.060	4
55	0.19607843	0.726	3
27	0.19607843	0.693	3
16	0.19230769	0.847	3

90	0.18975332	0.903	3
54	0.17857143	0.778	2
32	0.17182131	0.881	4
60	0.16949153	0.888	3
62	0.16949153	0.858	4
34	0.16129032	0.977	3
39	0.15847861	0.885	2
95	0	1.114	1
65	0	0.876	1
12	0	0.978	1
5	0	0.818	2
26	0	0.857	3
30	0	0.858	3
37	0	0.985	2
44	0	0.825	2
71	0	0.783	3
80	0	0.908	2
83	0	0.945	1
88	0	0.975	2
78	0	0.958	3

“El Mar es la encarnación de una existencia sobrenatural y maravillosa”- Jules Verne