Universitat Autònoma de Barcelona

Unravelling the (micro)plastic threat: the case study of plastic ingestion in Aristeus antennatus and Nephrops norvegicus from the NW Mediterranean Sea and its potential impact on health condition

Ester Carreras Colom

Universitat Autònoma de Barcelona

Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia

Unravelling the (micro)plastic threat:
the case study of plastic ingestion in *Aristeus antennatus* and *Nephrops norvegicus* from the NW Mediterranean Sea and its potential impact on health condition

A dissertation submitted by Ester Carreras Colom in fulfilment of the requirements for the degree of Doctor of Philosophy granted by the International Doctorate in Aquaculture in July 2021.

Supervisor and tutor

Maite Carrassón López de Letona

Universitat Autònoma de Barcelona

Supervisor

Joan Enric Cartes Rodríguez

Institut de Ciències del Mar (CSIC)

PhD candidate

Ester Carreras Colom

With the financial support of an FPU grant from the Spanish Ministry of Science, Innovation and Universities.

A la meva família

Acknowledgements/Agraïments

Ara que s'acaba aquest viatge, miro enrere i no puc més que somriure per l'experiència viscuda, per la gent que he conegut pel camí i per tots els moments viscuts. A totes les qui heu format part, d'una manera o d'una altra d'aquesta tesi, gràcies per fer arribar aquest vaixell a bon port.

En primer lloc, voldria agrair especialment als meus directors Maite i Joan per confiar en mi, sovint més del que jo vaig fer en mi mateixa.

A la Maite, per oferir-me l'oportunitat de formar part del seu laboratori amb aquest doctorat. Per guiar-me i animar-me sempre a treure el millor de mi. Per aconseguir que m'apassioni la ciència, ara més que mai. Sense tu res de tot això hagués sigut possible i t'estaré infinitament agraïda.

A en Joan, gràcies per introduir-me al món dels crustacis i per les teves valuosíssimes aportacions. Per ensenyar-me tant de la gamba vermella i el nostre mar i, sobretot, pel teu humor i la teva confiança en que tot aniria bé.

A tot l'equip de Veterinària, en majúscules, gràcies per haver-me fet sentir com a casa. Al Sito, per la teva ajuda inestimable en el camp, per mi totalment desconegut, de la histologia dels crustacis, per les discussions de passadís, per sempre veure-hi més enllà i, òbviament, per amenitzar les jornades amb música èpica. A l'Anna, per animar-me en aquell TFG fa ja tant temps, per declarar-te fan dels meus pòsters, per ser-hi tant present en aquesta recta final i en les batalles de plàstics i metalls, per regalar sempre un somriure. A la Maria, per introduir-me al món dels paràsits (tan de bo n'hagués trobat més!), pel teu ull crític, però sobretot, per ser una inspiració constant i una model a seguir. A la Sara, tot i haver coincidit poquet temps, per estar sempre disposada a oferir un cop de mà, per la paciència amb la bioquímica, per la teva serenitat.

A tu Oriol, que et mereixes menció especial, per ser el company infatigable d'aventures de la cara oculta de les nostres tesis. Pels cafès, els dinars i la multitud d'hores de discussions científiques, i no tant científiques. Senzillament, gràcies per ser-hi.

A la resta de companys de feina i alumnes de pràctiques que heu fet que les estones de laboratori fossin més entretingudes, però en especial a l'Encarna per donar-me un cop de mà sempre que ho he necessitat i a la Cèlia per tallar amb tant "carinyo" les gambetes.

To Natalie who was responsible for my stay in Dumfries at the University of Glasgow. Thanks for having me in your laboratory and trusting me for the experimental set up. I really spent three amazing months there.

A les companyes de viatge predoctoral, Anna Garriga i Ana Filipa, i la resta de companys d'unitat amb qui en algun moment hem compartit el dinar o el cafè, gràcies per ser un punt de trobada per agafar aire i continuar. Al Fernando, la Pilar, l'Òscar i el Manolo, per ajudar-me en les qüestions de logística i burocràcia que acompanyen el doctorat, la docència i les estades.

A totes aquelles que amb la vostra ajuda heu enriquit enormement aquesta tesi. A la Mireia, per acompanyar-me en els meus inicis en la identificació dels plàstics. A la Montse per acollir-me al seu laboratori i introduir-me en el món de la bioquímica. A la Cristina, per ajudar-me a entendre una mica més l'anàlisi dels metalls. També al Servei d'Anàlisi Química de la UAB i serveis cientificotècnics de la UB (CCitUB) sense els quals no hagués sigut possible posar el cartellet de plàstic al miler de fibres de colorin trobades.

A tots els pescadors i gent de mar que ha fet possible les campanyes, essencials per aquesta tesi. Ha estat una experiència inoblidable poder embarcar i formar part del vostre món momentàniament.

A la Mónica, en Pau, la Laura, l'Anna, la Berta, l'Aina i tot l'equip de Mustache, per aportar, de tant en tant, moments de calma i ajudar-me a recuperar forces entre riures.

A tu Andrea, pel teu amor i suport incondicional tots aquests anys. Gràcies per escoltar-me parlar dia rere dia del mateix, i seguir preguntant. Tenir-te al meu costat ha estat meravellós i espero que el nostre viatge no hagi fet més que començar.

Per últim, a la meva família, pares i germans, a vosaltres us ho dec tot. Per acompanyar-me en els èxits i fracassos i recordar-me que a l'Empordà sempre hi tindré un lloc. Gràcies per animar-me sempre a perseguir allò que em faci feliç.

Table of Contents

Abstract

The impacts of anthropogenic activities on marine environments are undeniable and are expected to increase in the near future. During the past decades, a particular form of pollution, plastics, has received great attention because of their persistence and widespread distribution. Their interaction with a wide range of marine organisms, mainly through entanglement or ingestion, has been reported, and experimental exposures have provided evidence for their potential negative impact. However, essential gaps in the knowledge of the sources, fate and impacts of plastics, particularly in the form of microplastics, still exist.

The Mediterranean Sea, and more particularly the Balearic Sea, has been highlighted as a potential site of plastic pollution accumulation, a hypothesis partially supported by evidence of high concentrations of plastic litter in surface waters. A significant proportion of these plastics is expected to eventually sink, thanks to biofouling and weathering processes, and reach the seafloor. Even though only a few studies have addressed the characterization of plastic pollution in the deep-sea environments to confirm it, they might eventually face high levels of plastic pollution.

Among all organisms inhabiting these environments, the decapod crustaceans *Aristeus antennatus* and *Nephrops norvegicus* might be more prone to ingest and retain plastics because of their close relationship with the sediment, their feeding mode, and a complex digestive system's morphology. Given their ecological relevance in bathyal assemblages and high commercial value, the study of plastic ingestion and its potential impact on these species is of particular interest.

The present thesis aims to characterise plastic ingestion, including microplastics, in the two important crustacean species of the Balearic Sea, the blue and red shrimp *Aristeus antennatus* and the Norway lobster *Nephrops norvegicus,* and to evaluate its potential impact on health condition of wild populations. Moreover, given the current needs for cost-effective indicators of plastic pollution in monitoring frameworks, another main goal will be to evaluate the potential use of these species as monitors of environmental levels of plastic pollution.

In the third chapter of the present thesis, levels of plastic ingestion in *Aristeus antennatus* from a broad spatial (including the continental and insular slopes) and depth (630-1870 m) range are reported. Despite slightly higher prevalence values were observed in areas close to Barcelona, a decreasing trend was not found with distance, thus pointing out the vast dispersion of plastics in deep areas. The potential role of the benthophagous diet and close relationship with the sea bottom of *A. antennatus* in the increased exposure and uptake of plastics is also discussed and further supported by observations in diet composition. Detailed analysis on prey's ecology revealed that shrimps with plastics had a higher contribution of endobenthic prey in recent meals. Despite some individuals showing aggregations of tangled up fibres, no correlations with shrimps' body condition indices were observed.

In the fourth chapter, plastic ingestion in *Aristeus antennatus* is further characterised in three selected locations along the Catalan coast with allegedly different anthropogenic sources for plastic pollution. Moreover, a temporal comparison (2007 vs 2017-2018) is performed for individuals sampled off Barcelona. Plastic fibres of varying lengths, colours and chemical compositions were observed in > 65% of the individuals analysed regardless of the location and sampling period. Many of these organisms showed aggregations of tangled up fibres, which reached diameters of up to 1 cm. Differences among locations were found in 2018, with greater fibre loads towards the south during spring, whilst in summer, shrimps from Barcelona showed a fibre load nearly thirty-times higher than those from other locations. Fibre load reported for shrimps in 2007 was comparable to that of shrimps captured in 2017 and 2018 (only spring), yet a shift in the proportion of acrylic and polyethylene terephthalate (polyester) polymers was detected. No consistent effects on shrimp's health condition were found either, except for the negative correlation between the gonadosomatic index and fibre load in shrimps off Barcelona in summer (location with the highest reported values of plastic ingestion).

In the fifth chapter, the levels and potential impact of plastic ingestion are assessed in the second objective species, *Nephrops norvegicus*. In this case, a battery of enzymatic biomarkers, condition indices and assessment of histological alterations are used to evaluate health condition of the individuals. The presence and characteristics of plastics in sediments and bottom water are also reported. Moreover, the potential impact of other toxic pollutants is also assessed by determining the tissue and environmental levels of trace metals. Ingested plastics, mostly fibres, were found in 86% of the individuals, with higher values in individuals from Barcelona. Analysis of environmental samples revealed similarities in the abundance and size distribution patterns between ingested fibres and fibres found in the water, although not in the polymer composition. Overall, plastic ingestion was not significantly correlated to changes in condition indices. However, two significant correlations were observed with enzymatic responses: a positive correlation with glutathione-S-transferase activity and a negative correlation with catalase activity, suggesting an increased potential oxidative response in those individuals with a higher load of ingested plastics. Levels of trace metals in musculature greatly varied among locations depending on the metal species, whilst in sediment samples, the higher concentrations of most metals were seen in the vicinity of Barcelona. A significant relationship with the relative body condition was not observed either, although the high concentration of organic As reported points out the need for a continuous monitoring programme for human consumption safety issues. Finally, and most importantly, the histopathological assessment did not reveal major alterations or pathologic conditions affecting the species in the area, thus suggesting that it may be able to cope with present pollutant levels.

Finally, in the sixth chapter, the potential use of tangled up fibres in stomach contents of *N. norvegicus* is discussed as an affordable indicator for environmental levels of plastic pollution. To do so, first, plastic presence and load are described in detail in individuals from several populations across the European distribution of the species. Then, after successfully relating the total load of plastics and the presence of balls, the prevalence of balls is used as a proxy for total plastic fibre ingestion in other locations. Overall, locations with increased levels of plastic ingestion (e.g., the Gulf of Cadiz, N Barcelona and, partially, the Clyde Sea) also demonstrated higher levels of prevalence of balls (up to 30% of the individuals), compared to other locations with lower values of plastic ingestion and even the complete absence of balls (e.g., the Ebro Delta area). Differences could not be attributed to differences in diet composition and were considered to reflect environmental differences. The prevalence of tangled balls of fibres, particularly in this species, is proposed as an affordable, cost-effective and easy to implement indicator of plastic ingestion in benthic organisms and, ultimately, of environmental levels of plastic pollution.

Resum

L'impacte de les activitats antropogèniques sobre el medi marí és innegable i es preveu que s'intensifiqui en un futur pròxim. Durant les últimes dècades, un tipus particular de contaminació, els plàstics, ha despertat una atenció especial per la seva persistència i distribució generalitzada. S'ha observat que poden interaccionar amb multitud d'organismes marins, principalment a través de la seva ingestió o perquè aquests hi queden atrapats. A més a més, exposicions experimentals han demostrat possibles impactes negatius. En general però, encara existeixen nombrosos buits en el coneixement de les fonts i el destí dels plàstics, així com dels seu potencial impacte, especialment en forma de microplàstics.

El mar Mediterrani, i més concretament la zona del Mar Balear, ha estat destacada com una potencial zona d'acumulació de plàstics, una hipòtesi parcialment recolzada per les altes concentracions de residus plàstics observades en aigües superficials. No obstant, és d'esperar que una proporció significativa d'aquests plàstics s'enfonsin gràcies al recobriment d'algues (*biofouling*) i processos de degradació, fent que arribin al fons del mar. Tot i que son pocs els estudis que de moment han caracteritzat la presència de plàstics al mar profund, es creu que aquests ambients poden arribar a presentar elevades concentracions de plàstics amb el temps.

D'entre tots els organismes que habiten aquests ambients, crustacis d'ambients batials com *Aristeus antennatus* i *Nephrops norvegicus*, podrien ser més propensos a la ingestió i retenció de plàstics a causa de la seva estreta relació amb el sediment, la forma en que s'alimenten i la complexa morfologia del seu sistema digestiu. Donada la seva importància ecològica en les comunitats batials i seu alt valor comercial, l'estudi de la ingestió de plàstics i el seu impacte potencial és d'especial interès en aquestes espècies.

La tesi actual té com a objectiu caracteritzar la ingestió de plàstics, incloent microplàstics, en *Aristeus antennatus* i *Nephrops norvegicus*, dues de les espècies de crustacis més importants del Mar Balear, i avaluar el seu impacte potencial sobre l'estat de salut de les poblacions salvatges. D'altra banda, un altre objectiu principal serà el d'avaluar l'ús potencial d'aquestes espècies com a indicadores dels nivells ambientals de contaminació per plàstics per tal de donar resposta a la falta d'indicadors de contaminació per plàstics en les xarxes de monitoratge actuals.

En el tercer capítol de la present tesi, es descriuen els nivells d'ingestió de plàstics d'*Aristeus antennatus* en un ampli gradient espacial (incloent-hi els vessants continentals i insulars) i de profunditat (630-1870 m). Tot i que es van observar uns valors de prevalença lleugerament més alts a les zones properes a Barcelona, no es va trobar una tendència decreixent amb la distància, assenyalant així la vasta dispersió dels plàstics en àrees de mar profund. També es discuteix el paper potencial d'una dieta rica en bentos i l'estreta relació amb el fons marí d'*A. antennatus* en l'elevada exposició i ingesta de plàstics, L'anàlisi detallat de l'ecologia de les preses va revelar que aquelles gambes amb plàstics presentaven també una major contribució de preses de l'endobentos als àpats recents. Tot i que alguns dels individus analitzats van mostrar agregacions de fibres embolicades (cabdells), en cap cas es van observar correlacions amb els índexs de condició corporal.

En el quart capítol, la ingesta de plàstics a *A. antennatus* es caracteritza en major detall a tres localitats seleccionades al llarg de la costa catalana sotmeses a diferents fonts antropogèniques de contaminació per plàstics. A més a més, es realitza una comparativa temporal (2007 vers 2017-2018) per a individus mostrejats a Barcelona. En global, es van observar fibres plàstiques de diferents longituds, colors i composicions químiques en > 65% dels individus analitzats, independentment de la seva ubicació i període de mostreig. Una part important d'aquests organismes van mostrar agregacions de fibres embolicades, amb diàmetres de fins a 1 cm. Les diferències observades entre les localitats mostrejades el 2018 van senyalar una major ingesta de plàstics cap al sud durant la primavera, mentre que a l'estiu, les gambes de Barcelona van presentar gairebé trenta vegades més plàstics ingerits que a la resta de llocs. A nivell temporal, la ingesta de plàstics en gambes del 2007 va ser comparable a les de gambes del 2017 i 2018 (primavera) en abundància, però es va detectar un canvi en la proporció de polímers d'acrílic i de tereftalat de polietilè (polièster). Tampoc es van trobar efectes consistents en la condició de salut de les gambes, a excepció d'una correlació negativa entre l'índex gonadosomàtic i les fibres ingerides observada en gambes capturades a Barcelona a l'estiu (zona amb els nivells d'ingestió de plàstics més elevats).

En el cinquè capítol, els nivells i l'impacte potencial de la ingestió de plàstic s'avaluen en la segona espècie objectiu, *Nephrops norvegicus*. En aquest cas, s'utilitza una bateria de marcadors enzimàtics, els índexs de condició i l'anàlisi d'alteracions histològiques per avaluar l'estat de salut dels individus. També es descriuen amb detall la presència i característiques dels plàstics en mostres de sediments i aigua. A més a més, l'impacte potencial d'altres contaminants tòxics (metalls pesants) també s'avalua tot determinant els nivells de concentració en organismes (musculatura) i al medi (sediment). Es van identificar plàstics ingerits, majoritàriament fibres, en el 86% dels individus, en major quantitat en aquells mostrejats prop de Barcelona. L'anàlisi de mostres ambientals va revelar similituds en els patrons d'abundància i distribució de mides entre les fibres ingerides i les presents a l’aigua, tot i que no pel que fa a composició de polímers. En general, la presència de plàstics no es va veure significativament correlacionada amb els canvis en els índexs de condició. No obstant, sí que es van observar correlacions significatives amb les respostes enzimàtiques: una correlació positiva amb l'activitat de la glutatió-S-transferasa i una correlació negativa amb l'activitat de la catalasa, suggerint un augment de la resposta oxidativa en aquells individus amb una major abundància de plàstics ingerits. Els nivells de metalls traça en musculatura van ser molt variables entre localitats de mostreig, mentre que en mostres de sediments, les concentracions més altes de la majoria de metalls analitzat van observar-se a Barcelona. En tot cas, tampoc es va observar una relació significativa amb els índexs de condició corporal tot i que davant les concentracions observades, seria recomanable realitzar un seguiment continu per a qüestions de seguretat alimentària. Finalment, l'avaluació histològica no va revelar alteracions importants o condicions patològiques afectant els organismes de la zona, suggerint així que podria ser capaç de fer front als nivells de contaminació actuals.

Finalment, en el sisè capítol es parla del possible ús de la presència de cabdells de fibres embolicades, trobats recurrentment en el contingut estomacal de *N. norvegicus*, com a indicadora dels nivells de contaminació per plàstics a l’ambient. Per fer-ho, primer es descriu la presència i abundància, així com les característiques dels plàstics ingerits en individus de diverses poblacions de la distribució europea de l'espècie. Llavors, després de relacionar amb èxit la càrrega total de plàstics i la presència de cabdells, s’utilitza la prevalença de cabdells per a l’estimació dels nivells d’ingestió de fibres plàstiques en altres localitats. En general, les ubicacions amb nivells més alts d'ingestió de plàstic (per exemple, el Golf de Cadis, Barcelona i, parcialment, el Mar de Clyde) també van demostrar nivells més alts de prevalença de cabdells (fins a un 30% dels individus), en comparació amb altres llocs amb valors més baixos d'ingestió de plàstic on, fins i tot, es va descriure la total absència de cabdells (per exemple, la zona del Delta de l'Ebre). Les diferències no es van poder atribuir a diferències en la composició de la dieta pel que es van considerar un reflex de les diferències en contaminació ambiental. Per tant, la determinació de la prevalença de cabdells de fibres, especialment en aquesta espècie, es proposa com un indicador assequible i fàcil d'implementar en el seguiment de la ingestió de plàstic en organismes bentònics i, en última instància, de la contaminació per plàstics a nivell ambiental.

CHAPTER 1 Introduction

1 Introduction

1.1 Plastic pollution: a new threat in a changing world

Human activities have an undeniable impact on Earth on a global scale. The increasing evidence of this profound impact has led to the recognition of a new geological era, the Anthropocene (Zalasiewicz et al., 2016). Human societies are driving a global and unprecedented change by extensively modifying the land surface and accelerating biodiversity loss and climate change, among other environmental changes (Barange et al., 2011).

Marine ecosystems have not been exempted from such human influence. Marine resources have been historically exploited for consumption and economic gain. However, population growth and technological advances led to the intensification of human impacts with major effects on the ecosystem. For example, sustained overfishing and the increasing power of fishing fleets led to the collapse of entire coastal ecosystems (Jackson et al., 2001). The historical perception of the sea as an enormous and immutable environment with an infinite recovery ability is finally starting to fade out.

Similarly, chemical pollution of aquatic environments received little attention until a threshold level was reached and adverse consequences started to appear (Shahidul Islam and Tanaka, 2004). Up until then, the sea was rather viewed as a site of waste disposal, a practice either justified by the dilution principle or deeply rooted in the "out of sight, out of mind" culture. Pieces of evidence on the impact of several chemical pollutants (e.g., heavy metals) have led to enhanced environmental protection. Nonetheless, with technological advances, the production and diversification of synthetic chemicals is increasing at an exponential rate and so might be their release into the environment (Bernhardt et al., 2017).

One of the most recent forms of pollution identified in marine environments have been plastics. Their persistence and ubiquity have raised the alarms of both the scientific and public communities as well as policymakers. Moreover, a particular form of plastic pollution, microplastics, has even attracted more attention. Because of their size, organisms might easily ingest them, which might allegedly lead to their bioaccumulation and therefore repeating the patterns observed for other relevant contaminants in the past.

Unlike other historic contaminants, such as heavy metals or persistent organic pollutants (POPs), plastics are easily distinguishable and are part of our lives on a daily basis. Despite being a diverse suite of contaminants of varied physical and chemical properties, plastic pollution, including microplastics, as a unitary concept has eclipsed the media and has become a “charismatic” threat (Battisti and Gippoliti, 2019). Surprisingly, regulations on plastic use, such as the ban on microbeads imposed in the United Kingdom in 2018, have even anticipated the scientific consensus on their negative impact on organisms or human health (Backhaus and Wagner, 2020; Burns and Boxall, 2018).

1.1.1 Brief history of (micro)plastics

The history of plastic dates back to humanity itself since ancient civilizations used natural plastics (i.e. resins) for varied purposes (Bijker, 1987). During the 1860s, the first man-made plastic, Parkesine, was manufactured from organic compounds, but it was not until 1907 that the first truly synthetic polymer, Bakelite, was created (Bijker, 1987; Reboul, 1997). During the period surrounding World War II, most of the major plastics we know today – polyethylene, polystyrene, nylon – emerged, and mass production of plastics started, mostly to meet military needs (Napper and Thompson, 2020).

Afterwards, the benefits of plastics for consumer products became obvious, and plastics started to take the place of traditional materials. Their adaptability, resistance and, most importantly, low price granted them the most advantageous materials for a wide range of applications. Thanks to plastics, major advances took place in various sectors, including healthcare, medicine, building or consumer technology (Andrady and Neal, 2009). As a result, global production of resins and fibres increased at an exponential rate.

The first observations of plastic litter in the ocean were recorded in the early 1970s, when plastic fibres and pellets were reported in surface waters (Buchanan, 1971; Carpenter et al., 1972; Carpenter and Smith, 1972; Heyerdahl, 1971). In particular, in their two works in *Science*, Carpenter et al. (1972) and Carpenter and Smith (1972) already raised the concern for these particles to be ingested by fish and cause intestinal blockage in small individuals as well as being a potential source of polychlorinated biphenyls (PCBs). These studies stimulated the interest in marine litter, and during the following decade, several studies focusing more extensively on the distribution of floating and beached plastics and their accumulation rate were published (Colton et al., 1974; Cundell, 1973). During the same period, attention also started to be placed on the seafloor, and the first studies reporting marine litter captured with fishing nets appeared (Holmström, 1975). Soon, the deep seafloor was identified as a potential site of accumulation of plastic debris, though still influenced by the proximity of potential sources and local hydrological conditions (Galgani et al., 1996, 1995; Goldberg, 1997).

In 2004, the publishing of "Lost at Sea: Where's all the plastic" (Thompson et al., 2004) marked a turning point. In that work, they pointed how a great number of microscopic plastic fragments might have resulted from the plastic (macro)litter originally released into the environment. They also demonstrated the broad spatial extent and accumulation that these microplastics had reach in a relatively short period of time. Together with the high media profile of the description of vast litter aggregations in the oceanic gyres (Eriksen et al., 2014; Law et al., 2010; Moore et al., 2001), Thompson et al. (2004) are considered responsible for the resurgence of interest in the marine litter problem and the popularization of microplastics (Bergmann et al., 2015).

During the past decades, hundreds, if not thousands, of works on plastic and microplastic pollution, mainly targeting marine environments, have been published (Lakshmi Kavya et al., 2020; Lusher et al., 2017). The presence of plastics has been reported in remote areas such as the depths of the Kuril-Kamchatka Trench (NW Pacific) (Fischer et al., 2015) or the deep Arctic seafloor (Bergmann et al., 2017), and their ingestion or entanglement has been documented for at least 914 marine megafauna species (Kühn and van Franeker, 2020; Provencher et al., 2017). Not to mention the wide range of experimental studies trying to prove whether plastics, mostly in the form of micro- or nano-plastics, may or may not have a negative impact on marine organisms and human health (Burns and Boxall, 2018; Rist et al., 2018).

It has been estimated that a total of 8300 million Mt of virgin plastic were produced as of 2015, of which 4900 Mt (~60%) would have already been discarded (Geyer et al., 2017) and

Jambeck et al. (2015) estimated that 4.8 to 12.7 million Mt entered the ocean in 2010 alone. Once plastics reach the environment, the same properties that granted their usefulness, low density (most plastics are positively buoyant) and high durability, enhance their rapid dispersion by water and wind (Ryan et al., 2009). They are now considered ubiquitous contaminants whose sources are strongly linked to human activities (**Fig. 1**). The increasing use of single-use products coupled with the inadequate disposal or poor waste management and recycling practices still enhance the arrival of these plastics to the environment. As a result, the levels of plastic, especially microplastic, pollution in marine environments worldwide are expected to keep increasing, as they have been in the past 60 years (Ostle et al., 2019).

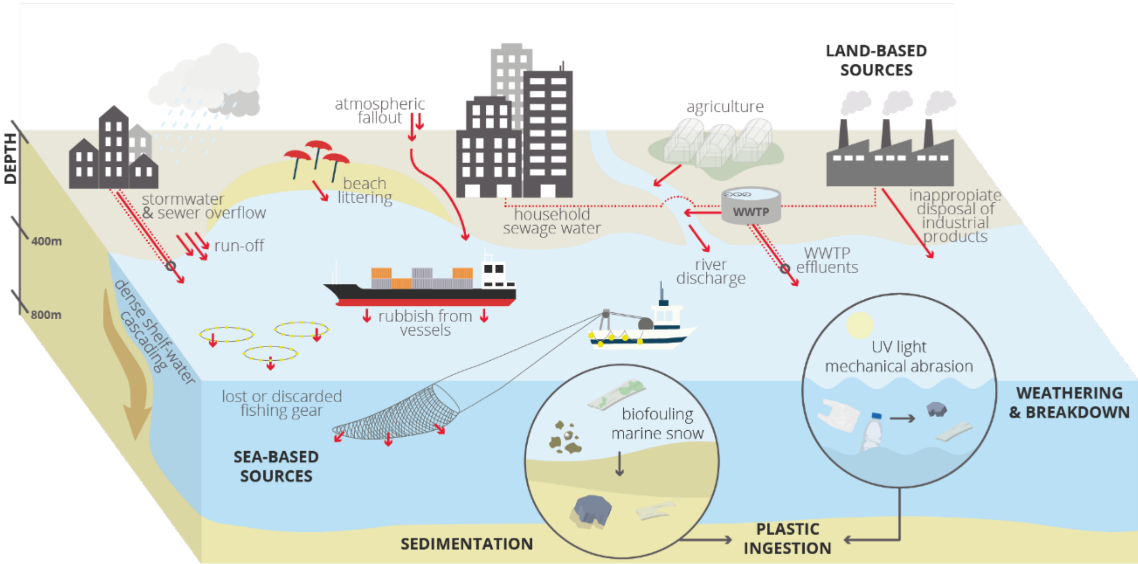


Fig 1. Main sources and pathways of plastic and microplastic litter into the environment.

1.1.2 Understanding the nature of microplastics

Rather than being a unique and clearly distinct substance, plastics are a diverse suite of items of varied characteristics (**Fig. 2**) and microplastics might be considered a subdivision of these contaminants with a particular size.

Chemical composition is, without doubt, the most fundamental criterion for defining plastic litter. According to the International Organization for Standardization, plastic is: “a material which contains as an essential ingredient a high polymer and which, at some stage in its processing into finished products, can be shaped by flow" (ISO, 2021). This definition leaves out elastomers, such as rubbers that might be easily mistaken for plastics, but includes a diverse range of polymers (polyethylene, polypropylene, polystyrene, polyvinyl chloride or polycarbonate, among many others) (Hartmann et al., 2019; Rochman et al., 2019). As simple as it may seem, confirming the plastic composition of items observed in the environment, particularly when facing particles of small sizes, requires the use of analytical methods such Fourier transform infrared (FTIR) or Raman spectroscopy (Käppler et al., 2015). Moreover, besides the main polymeric matrix, there can be a wide range of plastic additives added during the manufacturing process such as flame retardants, colourants, fillers, reinforcements, among others (Rochman et al., 2019).

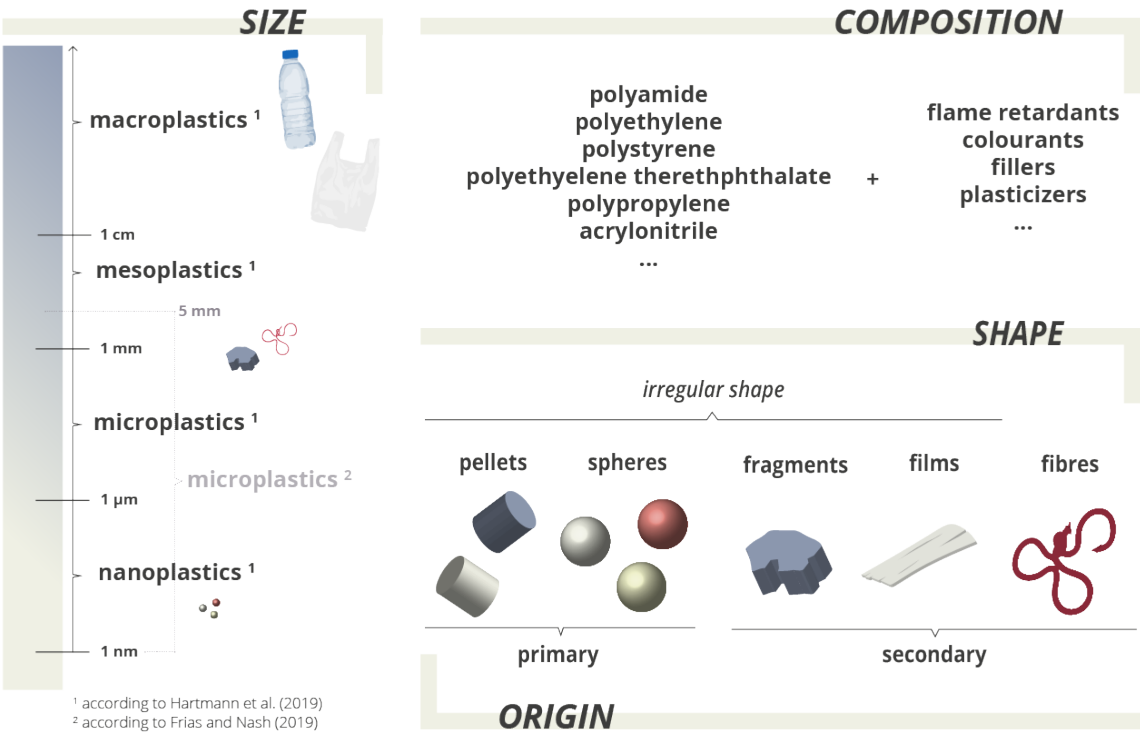


Fig 2. Common characteristics and criteria used in the description and classification of plastic debris.

Secondly, size has been used to discriminates among physically different forms of plastic pollution, e.g., to differentiate microplastics from plastic litter in a broad sense. Several definitions have been proposed for the term microplastic since it was first used by Ryan and Moloney (1990) to designate plastic artefacts of <20 mm of diameter (Hartmann et al., 2019). There is still some debate around its proper characterization, though there seem to be two main strands of thought regarding size limits. On the one side, there is a substantial number of studies that have agreed on the use of the definition of microplastics as particles smaller than 5 mm (Frias and Nash, 2019). This upper limit was set in some of the first studies focusing on microplastics (Arthur et al., 2009; Cole et al., 2011) and gained popularity through their recurrent use and adoption by international organizations (i.e. UN environment programme, NOOA or EFSA). On the other side, a recent line of research has proposed a more traditional approach based on the conventional units of size, defining microplastics as particles of 1 to 1000 µm (Hartmann et al., 2019). Overall, the problem with the definition of size limits extends to the lower boundary too, particularly now that there is a growing interest in differentiation between micro- and nanoplastics.

Finally, shape would be the third most used criterion when reporting levels of microplastics. Contrary to macro-debris, whose identity might be easily distinguishable and describable, when it comes to microplastics, difficulties arise in the description of shapes. For instance, terms frequently used are fragments, films, fibres, spheres, beads and pellets. The former two have been commonly used to represent particles of irregular shape, with films being considerably smaller in one dimension than in the other two. Thread-like structures with one dimension (length) considerably longer than the other two (width) are referred to as fibres. With fibres comes an example of how ambiguous the current definition of size is, e.g., what should a thin (less than 1-5 mm of diameter) but long (more than 1-5 mm of length) fibre be considered, a macro- or a micro-plastic? Hartmann et al. (2019) proposed to use the largest dimension as the classifier for size categories though countless examples exist on the classification of >1-5 mm long fibres as microplastics. Lastly, the terms spheres, beads and pellets have been often used as synonyms and rather than describing shapes, these terms imply the origin of the particles observed. Microplastics are considered to be either primary, intentionally manufactured in the size observed to be used, mainly as raw material in the plastics industry, or secondary, when they originate from larger plastic items (Browne et al., 2007; Cole et al., 2011). Secondary microplastics can originate from the wear and tear processes derived from the use of plastic products (i.e., the release of fibres from fabrics, tire wear particles) or from the weathering processes they experience once in the environment (Hartmann et al., 2019). Even though the strong bonds within the polymer and their high molecular weight grant them a high resistance to degradation, solar radiation, wave action, abrasion from sediment, or the interaction with organisms (e.g., biofouling, ingestion) can reduce the structural integrity of plastics and result in their fragmentation (Browne et al., 2007; Cole et al., 2011; Hodgson et al., 2018; Welden and Cowie, 2016a).

In addition to the varied criterion used for the identification of particles as plastics and their further categorization into different size categories, studies in this research field have also faced a recurrent debate on the methodologies to be used. In order to analyse and characterize the presence of plastics, a great variety of methods have been proposed, e.g., visual inspection, density separation, digestion followed by filtration (Karlsson et al., 2017; Lusher et al., 2017; Rocha-Santos and Duarte, 2015; Silva et al., 2018). And for each of them several detailed protocols can be found in the literature using different reagents or detailed protocols (e.g., digestion procedures with acids, bases or enzymes; Enders et al., 2017; Kühn et al., 2017). Overall, there is no broad consensus on the best methods for plastics analysis, which may in fact depend on the particular aim of each study. For instance, visual inspection might be suitable for the monitoring of plastic entanglement in marine turtles or microplastic ingestion but might not be suitable for the characterization of nanoplastics in digestive contents (Hidalgo-Ruz et al., 2012; Renner et al., 2018). However, as a result, values reported in different studies are sometimes hardly comparable and reproducible (Cowger et al., 2020).

1.1.3 The potential impact of (micro)plastics

The possibility that plastics may have an impact on organisms has arisen from the simple fact that they have become widespread in the marine environment. When trying to understand their potential impacts on marine biota, it is key to account for the diversity within plastics and microplastics. As mentioned above, they should be viewed as a diverse contaminant suite whose different characteristics, including their polymer composition, size and shape, as well as other less addressed characteristics such as their colour or the presence of additives (Rochman et al., 2019), might play a key role on modulating their potential impacts.

Mechanical effects of plastics, mainly entanglement and ingestion, were the first to be noticed already in the 1980s (Laist, 1987). Records of entanglement of marine wildlife have continued to date. They now include a long list of marine mammals, sharks, turtles, and seabirds that have been observed entangled to plastic macro-debris such as plastic bags, lost or discarded fishing nets and a wide variety of consumer goods (e.g., the plastic six-pack harnesses used to hold drink cans) (Baulch and Perry, 2014; Colmenero et al., 2017; Derraik, 2002; Duncan et al., 2017; Laist, 1987; Ryan, 2018). Entanglement may significantly affect the ability to move, feed and breathe, and eventually lead to the individual's death (Baulch and Perry, 2014). Marine megafauna has received the most attention in the matter, but smaller organisms, e.g., fish and invertebrates, may be expected to suffer similar impacts. For instance, ghost fishing could be viewed as a particular case of plastic entanglement for fish (Brown and Macfadyen, 2007).

Despite being less visible than entanglement and requiring a greater effort to characterise it, plastic ingestion is probably the most reported interaction in field studies between plastic pollution and marine biota. Varying levels of plastic ingestion in terms of prevalence and abundance have been reported among marine organisms, a variability that has been mainly attributed to environmental differences, meaning different levels of exposure, and intrinsic factors such as the feeding behaviour (Avio et al., 2020). It has been hypothesised that some organisms, mainly visual predators, may actively ingest plastics because of their resemblance with natural prey. The observations of benthic phase turtles showing a strong selectivity for soft, clear plastic that might have been mistaken for jellyfish (Schuyler et al., 2012) or those provided by Ory et al. (2017) on the great prevalence of blue polyethylene fragments ingested by *Decapterus muroadsi* (Carangidae) whose diet commonly included copepods very similar in size and colour to said fragments, would provide further support to the hypothesis of active feeding on plastics.

On the other side, passive ingestion has also been suggested as an important route for plastic uptake in marine organisms, with plastics being potentially ingested with prey (or in prey itself, which could be considered trophic transfer) from the sediment or while suspended in the seawater (Browne et al., 2007; Cole et al., 2011). As a consequence, those organisms whose food sources overlap with the distribution of higher concentrations of plastics, i.e., benthic feeders, or whose feeding strategy might be more susceptible to ingest plastics, i.e., filter-feeders and deposit-feeders, may show an increased plastic uptake (Karlsson et al., 2017; Wright et al., 2013b). Planktivorous and benthic fish, as well as benthic crustaceans and invertebrate deposit- and filter-feeders, have been confirmed to ingest plastics in natural settings (Avio et al., 2020; Li et al., 2016; Markic et al., 2020;

Murray and Cowie, 2011; Taylor et al., 2016). Anastasopoulou et al. (2013) related the presence of hard items of plastics to certain fish species' bathybenthic feeding habits, and Avio et al. (2020) reported a higher diversity of polymers in benthic and pelagic species. In the latter, a higher frequency of microplastic ingestion was observed in benthopelagic species than pelagic and benthic ones, which was attributed to their higher possibilities to interact with microplastics from the two compartments. Other opposing results can be found in the literature, with Karlsson et al. (2017) reporting the highest concentrations in filter-feeders, while Bour et al. (2018a) did not observe any influence of the feeding mode on the levels of ingested microplastics. Overall, the scarcity of studies simultaneously evaluating levels of plastic ingestion in organisms with different feeding strategies and environmental concentrations hinder the proper evaluation of feeding strategy or trophic transfer as significant factors for increased plastic ingestion.

Regardless of the route of entry, once inside the digestive tract, plastics may have a different effect depending on their size in relation to the organism's size. They can result in physical impacts such as perforations or internal abrasions and even lead to gut obstructions if they are big enough (Gregory, 2009; Markic et al., 2020; Wright et al., 2013b). Also, plastics may be more or less easily expelled depending on their size, shape and the organism's digestive system's morphology. The level of obstruction will likely depend on the size of the item ingested, which must be small enough to be available for ingestion, and big enough to produce such blockage at some point of the digestive tract. Something to be taken into account is the flexible and elastic nature of some polymers that may ease the ingestion of larger items, e.g., latex balloons, polyethylene bags. In other cases, the aggregation of plastic items once ingested would also hinder their expulsion. For instance, Murray and Cowie (2011) described the presence of large aggregations of fibres in the stomach contents of *Nephrops norvegicus,* and later, Welden and Cowie (2016a) reported the presence of similar aggregations after the exposure to isolated fibres under controlled conditions. In the latter case, the prolonged and increased exposure to plastic fibres due to them being retained in the stomach was associated with a reduction in the body condition of langoustines. This reduction was further attributed to a false satiation effect caused by the volume taken by plastics that led to a reduced feeding activity and, ultimately, a decrease in the nutritional state.

Since plastics have little (if they show signs of algae growth or epibionts) to no nutritional value, the potential impact of plastic ingestion on the physical condition through the reduction in the feeding stimulus has been long discussed (Derraik, 2002). Other feasible implications might be reduced growth rates, diminished predator avoidance, lowered steroid hormone levels or delayed ovulation and reproductive failure (Wright et al., 2013b). Even though little evidence has been found in wild populations, see, for example, the negative correlation sporadically observed between plastic ingestion and body condition in the rocky shore crab *Pachygrapsus transversus* (de Barros et al., 2020) or between the presence of plastics and stomach repletion in the Narwal shrimps *Plesionika narval* (Bordbar et al., 2018), experimental exposures have provided strong support to this hypothesis. For instance, following controlled exposures, marine worms and sediment-dwelling bivalves displayed decreased energy reserves (Bour et al., 2018b; Wright et al., 2013a), and green shore crabs showed a reduced scope for growth (Watts et al., 2015).

The possibility of translocation into the cell and bioaccumulation of plastic pollutants is one of the main current challenges for the scientific community. To date, no clear biomagnification trends have been observed in the field (Miller et al., 2020), although translocation across the gastro-intestinal membranes and distribution into tissues and organs has been demonstrated under controlled conditions for particles situated in the lowest size range of microplastics and nano-plastics (Jeong et al., 2016). Regardless of the potential internalization of the plastic agents used, experimental exposures to small-sized microplastics have shown a number of negative outcomes in fish and invertebrates, including oxidative stress, compromised immune response and tissue damage (e.g., splitting of enterocytes) (Brandts et al., 2018a, 2018b; Lei et al., 2018). One of the future challenges will be to test whether nanoplastics might show significant impacts in the field as well as to estimate the potential contribution of ingested microplastics to the release and presence of nanoplastics in digestive contents.

Beyond their potential impact as particles, the role of (micro)plastics in the chemical transfer of plastic additives and other hydrophobic organic chemicals (HOCs) to marine organisms has also been discussed. On the one hand, leaching of plastic additives would be related to the degradation of plastics themselves, leading to the release of substances that were added to the virgin plastic material during its manufacture such as phthalates, bisphenol A or polybrominated diphenyl ethers (Browne et al., 2013; Koelmans et al., 2014).

Some of these substances are biologically active and may play a more prominent role than plastics as particles in the impact on marine biota (Bergmann et al., 2015; Koelmans et al., 2014; Meeker et al., 2009).

On the other hand, another line of research has been devoted to investigating the potential role of plastics as vectors of HOCs. Thanks to their strong binding properties for HOCs, especially microplastics for their higher surface to volume ratio, it has been suggested that plastics might have a propensity to sorb and transfer to biota these substances (Rochman et al., 2013; Teuten et al., 2009). Further studies have demonstrated that HOCs are present in microplastics recovered from the field and can be successfully transferred to biota under experimental conditions. However, a recent study provided evidence that the fraction of HOCs sorbed by plastics would be small compared to other media like prey (Koelmans et al., 2016).

In broad terms, and even though some significant impacts have been recorded under experimental exposures, there is no clear consensus on the impacts of microplastics *per se*, especially when considering the size range >20μm (Burns and Boxall, 2018; Foley et al., 2018; Jacob et al., 2020). A common criticism on the experimental exposures to microplastics conducted to date has been the use of environmentally unrealistic exposures, be it because: (1) concentrations used were orders of magnitude higher than the concentrations reported in the environment, (2) the plastics used roughly represented the diverse typologies of plastics observed in the wild, i.e., virgin plastics composed of only one type of polymer and representing a single size and shape, or (3) plastic exposures were tested against particle-free treatment exposures (Backhaus and Wagner, 2020; Lenz et al., 2016). Moreover, most of the studies have focused only on very specific indicators of impact, whilst multidisciplinary approaches would be strongly advised since we do not completely understand the dynamics of microplastics yet. Not to mention that laboratory experiments can hardly reproduce the multifactorial stressors that wild organisms might be facing.

Now more than ever, there is an imperious need for the proper assessment of the potential impact of (micro)plastic pollution on marine organisms' health in the wild with current levels of exposure, as well as the establishment of monitoring networks based on the use of cost-effective indicators that allow for the identification of areas of particular concern. Given that plastic ingestion is common in marine biota and that it is expected to be related to environmental levels to some extent, levels of plastic ingestion in certain species might be used as indicators of plastic pollution. A successful case of study is the use of northern fulmars (*Fulmar gracilis*) to monitor plastic litter in the North Sea area (van Franeker et al., 2011). However, to date, no marine species have been selected to monitor microplastic pollution on smaller spatial scales, and most analytical methods for the identification of microplastics are considered too time-consuming to be readily applied on existing monitoring frameworks (Bonanno and Orlando-Bonaca, 2018; Fossi et al., 2017).

1.2 The Mediterranean Sea: a hotspot for anthropogenic impacts

The Mediterranean Sea has been a historically populated area, particularly along their coastlines where, ever since the ancient civilizations, urban settlements have thrived. Intense industrial, urban, and agriculture uses, together with the release of their associated chemical pollutants, heavy marine traffic and important fishing industries along their continental shelves, have put the Mediterranean marine ecosystems under tremendous cumulative pressure and future scenarios point to a significant increase in the risks associated to these impacts in the coming decades (Cramer et al., 2018; MedECC, 2020). It has been estimated that over 20% of the entire basin and nearly the totality of the territorial waters of the EU member states are heavily impacted (Micheli et al., 2013). In fact, it is thought that the Mediterranean Sea may be experiencing the global trends in climate and environmental change to a larger extent due to the high anthropogenic pressure. For instance, its narrow shelves and the decline in the fish stocks motivated the shift of the fishing fleet towards deeper regions a long time ago, and Mediterranean waters are warming approximately 0.4 above the global average (MedECC, 2020 and references therein).

On top of that, it has been identified as a hotspot for plastic pollution with levels of surface plastic and microplastic debris equivalent to that identified in the oceanic gyres (Cózar et al., 2014; Suaria et al., 2016; Suaria and Aliani, 2014). The densely populated coastlines and intense industrial and agricultural uses, as well as the influence of rivers with heavily urbanised watersheds (e.g., Nile, Rhone, Po), might be acting as great sources of plastic litter, which then coupled to the hydrodynamics of a semi-enclosed basin might explain the rising levels of plastic pollution in the area.

Given that the Mediterranean Sea harbours between 4 and 25% of the world's marine species (Coll et al., 2010), plastic pollution, in conjunction with all the anthropogenic impacts present in the area (i.e. fishing, habitat loss, other forms of chemical pollution, climate change, eutrophication), must be regarded as a particularly concerning threat to the marine wildlife of the area.

1.2.1 Bathyal decapod crustaceans from the Balearic Sea: threatened by (micro)plastic pollution?

The Balearic Sea is situated in the north-western Mediterranean, delimited by the Spanish coastline and the Balearic Islands (**Fig. 3**). Modelling approaches have pointed it out as one of the main potential areas for plastic accumulation in the near future (Coppini et al., 2018; Kaandorp et al., 2020; Liubartseva et al., 2018), and several studies have already highlighted the current levels of microplastics in surface waters (Camins et al., 2020; de Haan et al., 2019; Ruiz-Orejón et al., 2018). Deeper areas have been less studied regarding microplastics, but marine litter, mainly in the form of derelict fishing gear partially made of plastic, has also been extensively recorded in the continental shelf and submarine canyons (Galgani et al., 1995; Galimany et al., 2019; García-Rivera et al., 2018; Tubau et al., 2015). Moreover, deep-sea sediments are regarded, in general terms, as a major sink for microplastics (Sanchez-Vidal et al., 2018; Woodall et al., 2014). Even though most plastics are considered to be positively buoyant, thanks to biofouling and adherence of particles as well as the action of waves, they become negatively buoyant and eventually sink.

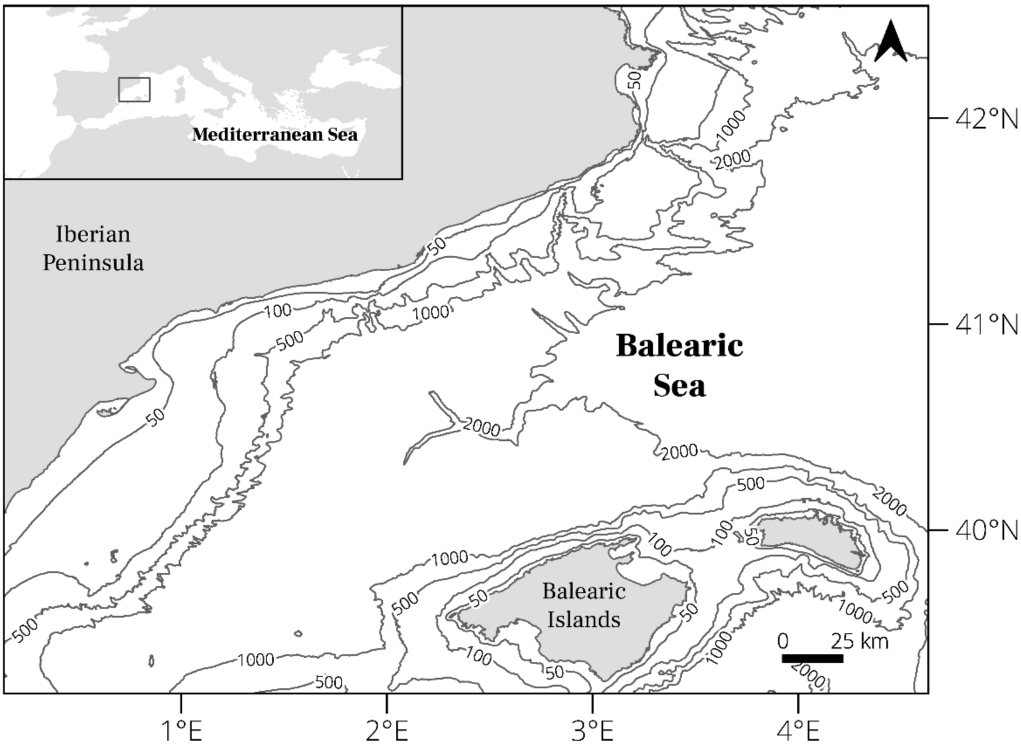


Fig 3. Map of the study area. Depth contours reprinted from EMODnet Bathymetry Consortium (2020).

Therefore, it is highly likely that organisms inhabiting the deep-sea sediments of the area might be exposed to great levels of plastic pollution. Among these organisms, and based on the evidence provided from worldwide field studies and laboratory experiments, benthic crustaceans might be one of the groups more prone to ingest, as benthic feeders exposed to plastics accumulated in the sediment, and retain, through the aggregation of ingested items, great values of plastics, such as those repeatedly observed for *Nephrops norvegicus* in the Clyde Sea (Murray and Cowie, 2011; Welden and Cowie, 2016c). Since most decapod crustaceans are expected to have a similar digestive system (Patwardhan, 1935, 1934), the formation of such aggregations could be expected in other representatives.

In the Balearic Sea, benthic crustaceans are an important part of the megafaunal assemblages. The dominance of decapods among invertebrates in Mediterranean waters greatly contrasts with the dominance of echinoderms in Atlantic waters at a similar latitude (Cartes and Sardà, 1993). As a result, they have been an important resource for the local commercial fisheries. Among all species, *Nephrops norvegicus* and *Aristeus antennatus* represent probably the most economically and ecologically relevant species in the Balearic Sea (Cartes et al., 1994; Cristo and Cartes, 1998; Gorelli et al., 2017). They are both the dominant decapod crustaceans from their respective assemblages, and even though they are not considered top predators, they occupy a high position in the trophic chain. Overall, besides the potential impact of plastic ingestion, these species might also be experiencing the impact of other environmental stressors such as fishing and the potential bioaccumulation of other chemical pollutants known to occur in the area, e.g., heavy metals (Palanques et al., 2017).

1.2.2 The blue and red shrimp, *Aristeus antennatus* (Risso, 1816)

Aristeus antennatus (Decapoda: Aristeidae) is a demersal deep-sea shrimp distributed throughout the Mediterranean Sea and in Atlantic waters from Cape Verde to the Portugal Coast (Crosnier and Forest, 1973; Lagardère, 1977). In the Mediterranean, it has a mid-bathyal distribution ranging from 450 to 3300m with the highest abundances observed between 600 and 1200 m (Cartes et al., 2017). Its diet consists of a great diversity of benthic and benthopelagic organisms, with ontogenic changes in diet and an intimate relationship with submarine canyons (Cartes, 1994; Cartes et al., 1994). Thanks to its great commercial interest, a great number of studies have been devoted to its diet (Cartes, 1994; Cartes et al., 2008; Cartes and Sardà, 1989), sexual development and recruitment (Carbonell et al., 2008; Cartes et al., 2017; D'Onghia et al., 2009; Demestre et al., 1997), moult (Sardà and Demestre, 1985), and other aspects of its biology and ecology (Carbonell et al., 2010, 1999).

Ingestion of plastic debris by *A. antennatus* (i.e., fishing line reported as "nylon threads") was noted sporadically in diet studies conducted in the Balearic and Ionian seas (Cartes et al., 2008; Kapiris and Thessalou-Legaki, 2011), but its prevalence and potential impact have never been properly addressed to date.

1.2.3 The Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758)

Nephrops norvegicus (Decapoda: Nephropidae) is a benthopelagic crustacean with a wide geographical distribution, from the north-west Atlantic coasts to the eastern Mediterranean Sea (Zariquey Alvarez, 1968). Its bathymetric distribution in the Mediterranean reaches depths of 870 m, although its maximal densities have been found between 245 and 485 m (Cartes et al., 1994). This species is considered a generalist predator and scavenger whose diet includes crustaceans, echinoderms, polychaetes, molluscs, foraminifera and fish (Cristo, 1998; Johnson and Johnson, 2013). A particular feature of the species is its burrowing behaviour as individuals excavate extensive burrows to provide refuge from predators. In particular, egg-bearing females would spend prolonged periods of time inside the burrows (Rice and Chapman, 1971).

Similarly to *A. antennatus*, its great commercial and environmental value has encouraged numerous studies since the 1980s both in the Atlantic and Mediterranean areas to characterise its biology and ecology, including diet, growth, ecosystem roles, social behaviour or reproduction patterns, among others (see Johnson and Johnson (2013) and references therein). Of particular interest is the occurrence of epibiotic symbionts and pathological conditions reported in populations of *N. norvegicus,* especially in the Clyde Sea, whilst almost none of them have been reported in Mediterranean waters to date (Stentiford and Neil, 2011).

Concerning plastic pollution, several studies have characterised the levels of plastic ingestion in wild individuals from the Clyde Sea (Murray and Cowie, 2011; Welden and Cowie, 2016c), the Irish coast (Hara et al., 2020; Pagter et al., 2020), the Balearic Sea (Alomar et al., 2020) and the Adriatic Sea (Avio et al., 2020; Cau et al., 2019). Moreover, experimental studies have been conducted under controlled conditions to explore the effects of prolonged exposure to plastic fibres (Welden and Cowie, 2016b) and to evaluate the potential of microplastics as vectors of PCBs (Devriese et al., 2017).

1.3 Assessing the potential impact of pollutants on health condition

Assessing the health status of organisms can be very challenging in natural settings where organisms may be subject to multifactorial impacts. Although there is no single biomarker that may unequivocally measure environmental degradation, when combining markers of effects at different levels, we might be able to point out trends potentially related to the biological response to polluted environments (Galloway et al., 2004) and even detect early signs of impairment in populations (Colin et al., 2016).

Some of the most commonly used techniques for health assessment in marine organisms are the use of condition indices, biochemical determinations of enzymatic activities, and the histopathological assessment of target organs.

Body condition indices, including the relative condition factor and the hepatosomatic and gonadosomatic indices, are measures of body mass (i.e., "plumpness") relative to size. They are considered an estimate of the nutritional status or fitness, which may result from past foraging success, feeding intensity or exposure to environmental stress (Jakob et al., 1996; Jones and Obst, 2000). However, given the multiple confounding factors that exist (e.g., genetic differences, variable relationship with lipid content or reserves, seasonal trends associated with reproductive changes), when used with no other fitness measures, they should be carefully interpreted (Wilder et al., 2016).

The determination of multiple enzymatic activities makes it possible to integrate effects occurring at several physiological pathways (Crespo and Solé, 2016). For instance, cholinesterase activities are widely used as indicators of exposure to chemical contaminants (i.e., organophosphorus pesticides) (Fulton and Key, 2001), whereas antioxidant enzymes such as catalase have been related to oxidative stress (Valavanidis et al., 2006; Winston and Di Giulio, 1991). In any case, there is a body of work supporting their use in the evaluation of the exposure to environmental pollutants, including plastics, in a wide range of organisms (Koenig et al., 2013; Solé et al., 2010b, 2010a; Suman et al., 2021).

Finally, histological assessment has proved useful to evaluate the biological effects of contaminants in fish (Feist et al., 2015; Stentiford et al., 2003). Even though significant changes have been reported, for example, in the hepatopancreas of penaeid shrimps exposed to hydrocarbons, the usage of histological techniques to assess pollution trends in crustaceans has been less extended (Sreeram and Menon, 2005). In crustaceans, though, histopathological assessment has been extensively used to evaluate and characterise parasitic infections (e.g., *Hematodinium* sp. or bacterial infections) and other pathological conditions (Manan et al., 2015; Stentiford and Neil, 2011). Moreover, the prevalence and level of parasitic infections may also be used as indicators of stress conditions, pollution gradients and altered host's response (Marcogliese, 2005). Histological assessment has even proved useful in nutritional assessments (Vogt et al., 1986).

Each of these approaches covers a different temporal scale and type of response to environmental stressors (including pollutants). Enzymatic determinations mostly indicate early responses to toxic substances at a sub-individual level and, therefore, might have a small ecological relevance if these responses do not translate into significant effects at the individual level. On the other side, histological alterations might be considered intermediate responses at an individual level of greater ecological relevance since they result from adverse biochemical and physiological changes that have led to discernible tissue alterations or diseases. Condition indices could be regarded as markers of physiological state halfway through enzymatic activities and histological alterations in terms of response and ecological relevance. Therefore, when used all together, these markers might help elucidate whether potential effects of a toxic agent are limited to a sub-individual level or have rendered changes in upper levels of organization, e.g., histopathological alterations, meaning they might have a more relevant environmental impact.

CHAPTER 2 Objectives

2 Objectives

Considering the insufficient knowledge on the effects of (micro)plastic pollution on marine organisms in the field as well as the raising evidence for their accumulation in the Mediterranean deep sea, the primary purpose of the present thesis is to characterise (micro)plastic ingestion in two important bathyal crustacea species of the Balearic Sea and evaluate its potential impact on health condition of wild populations. Another main objective is to evaluate the potential use of plastic ingestion in these species as an indicator of environmental plastic pollution.

In order to fulfil these general goals, the following specific objectives are defined:

1) To determine the levels of plastic ingestion in wild populations of key crustacea species of the NW Mediterranean Sea, *Aristeus antennatus* and *Nephrops norvegicus*.

2) To provide an accurate characterization of (micro)plastics ingested in size, shape, and chemical composition through optical microscopy and FTIR.

3) To analyse the spatial and temporal variability in the prevalence and abundance of (micro)plastics ingested and discuss the trends observed as a function of potential environmental (i.e., potential sources) and biological (i.e., sex, size or moult stage) drivers.

4) To analyse the feeding intensity and diet composition and its relationship with ingested (micro)plastics to infer the most likely route of (micro)plastics uptake.

5) To evaluate the potential use of (micro)plastic ingestion in these species as an indicator for environmental levels of (micro)plastic pollution.

6) To determine the presence and abundance of (micro)plastics and their characteristics (i.e., size, shape, chemical composition) in bottom water and sediment matrices to establish the correlation between environmental exposure levels and organisms' ingestion.

7) To analyse the relationship between general condition indices (i.e., relative condition factor, hepatosomatic index and gonadosomatic index) indicative of general nutritional status as a proxy of health status and the prevalence and abundance of ingested (micro)plastics.

8) To perform histopathological analyses of target organs (i.e., gills, hepatopancreas, gonad and abdominal muscle) in order to detect the presence of pathological conditions potentially related to (micro)plastic ingestion or other pathological conditions (i.e., parasitic infections) that might modulate organisms' response to other stressors.

9) To determine enzymatic activities known to relate with potential biological and stressing conditions (i.e., lactate dehydrogenase, citrate synthase, acetylcholinesterase, carboxylesterase, glutathione-S-transferase, catalase, ethoxyresorufin-O-deethylase) and relate them with levels of (micro)plastic ingestion or other potential pollution gradients.

10) To integrate the variability of environmental parameters and levels of other toxic pollutants (e.g., heavy metals) in the health assessment.

CHAPTER 3 Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*

Marine Pollution Bulletin 133 (2018) 44–52

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*

Ester Carreras-Colom[a], Maria Constenla[a], Anna Soler-Membrives[a], Joan E. Cartes[b], Mireia Baeza[c], Francesc Padrós[a], Maite Carrassón[a,*]

[a] Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain
[b] Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain
[c] Departament de Química, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain

A R T I C L E I N F O

Keywords:
Microplastic ingestion
Fibers
Mediterranean Sea
Deep Sea
Aristeus antennatus

A B S T R A C T

Microplastic (MP) ingestion has been reported in a wide variety of organisms, however, its spatial occurrence and effects on wild populations remain quite unknown. The present study targets an economically and ecologically key species in the Mediterranean Sea, the shrimp *Aristeus antennatus*. 39.2% of the individuals sampled had MP in their stomachs, albeit in areas close to Barcelona city the percentage reached values of 100%. Overall, MP ingestion was confirmed in a wide spatial and depth (630–1870 m) range, pointing out the great dispersion of this pollutant. The benthophagous diet and close relationship with the sea bottom of *A. antennatus* might enhance MP exposure and ultimately lead to accidental ingestion. Detailed analysis of shrimps' diet revealed that individuals with MP had a higher presence of endobenthic prey. Microplastic fibers are probably retained for long periods due to stomach's morphology, but no negative effects on shrimp's biological condition were observed.

1. Introduction

Plastic pollution has attracted increasing attention worldwide during the last decade and it is currently one of the most concerning threats for wildlife and natural resources (Law and Thompson, 2014). In particular, there is a rising concern about microplastics (MPs), here defined as particles of < 5 mm in size (GESAMP, 2015). They can be of primary origin (i.e. manufactured with this size) or originate from the fragmentation of larger items of plastic (Lusher et al., 2017).

Microplastics can be dispersed over long distances due to their buoyant and persistent properties coupled with surface tension and oceanographic currents. Moreover, the biofouling process and formation of marine snow, make them eventually settle and spread also vertically through the water column and reach the seabed level (Claessens et al., 2011; Tubau et al., 2015; Woodall et al., 2014). As a consequence, they have already been found in a variety of marine habitats, from shorelines (Ryan et al., 2009) to deep waters (Taylor et al., 2016; Woodall et al., 2014) and even in the remote Antarctic marine system (Waller et al., 2017), with low human activity.

Because of their small size, MPs are bioavailable to a wide range of organisms, especially through their ingestion in organisms situated at low trophic levels. Overall, MP ingestion has been confirmed for over 220 species (Lusher et al., 2017). Many of these reports include the uptake of MPs by invertebrates, with high values of contaminated individuals in crustaceans (Devriese et al., 2015; Murray and Cowie, 2011). Once inside the organisms, MPs can lead to multiple damages, from direct physical harm such as attachment to the digestive structures, internal abrasions or digestive blockage to secondary detrimental effects on overall condition or reduced fecundity, among others (Browne et al., 2015; Law and Thompson, 2014; Wright et al., 2013a). Most of these evidences have been tested in experimental designs, yet effects of MP ingestion in natural environments are still poorly known (Cesa et al., 2017; Wright et al., 2013b).

In the case of the Mediterranean Sea, given its unique ecosystems and historically high pressure of anthropogenic activities, impact of MPs could be particularly relevant (Cózar et al., 2015). It has already been identified as a region of particularly high plastic litter concentration (Lebreton et al., 2012; Woodall et al., 2014), with an average value of 423 g km^{-2} and 1 item m^{-2} in surface waters that can be compared to that in the accumulation zones of the five subtropical gyres (Cózar et al., 2015). These values could be explained by both human and environmental factors. Its densely populated and industrialized coastlines, together with the discharge of important rivers such as the Rhône River in the Western Mediterranean Sea, and a high maritime

* Corresponding author.
E-mail address: Maite.Carrasson@uab.es (M. Carrassón).

https://doi.org/10.1016/j.marpolbul.2018.05.012
Received 13 April 2018; Received in revised form 6 May 2018; Accepted 7 May 2018

traffic, would explain a great input of marine litter (Eriksen et al., 2014; Tubau et al., 2015). This, coupled with its small area and the hydrodynamics of a semi-enclosed basin, which means that there is a small export of litter to the Atlantic Ocean through the Strait of Gibraltar, explains the great accumulation of plastic debris in the Mediterranean Sea, not only at the surface but also at seabed levels (Cózar et al., 2015; Tubau et al., 2015). The vast majority of research concerning MP ingestion in the Mediterranean Sea has focused on fish species (Alomar and Deudero, 2017; Anastasopoulou et al., 2013; Bellas et al., 2016; Cartes et al., 2016; Collard et al., 2017; Nadal et al., 2016; Romeo et al., 2015) but no studies have been properly addressed with crustaceans as study species.

The deep-water shrimp *Aristeus antennatus* (Risso, 1816) is a commercially and ecologically relevant species (Cartes et al., 2017, 2008) dominant in deep-sea communities (Cartes and Sardà, 1993). It inhabits mainly the western and central Mediterranean basins (D'Onghia et al., 2005) and has a strong relationship with submarine canyons since a trophic point of view (Cartes, 1994), with reproduction and especially recruitment taking place at depths > 1000 m, below fishing grounds (Cartes et al., 2017; D'Onghia et al., 2009). Despite having a wide bathymetric distribution, from 600 to 3300 m in the western and central basins (Sardà et al., 2004), highest abundances have been described at depths between 600 and 1200 m (Cartes et al., 2017; D'Onghia et al., 2009). Moreover, this species is one of the most valuable and targeted demersal resources in the Balearic Basin and their catches by bottom trawlers make up for 50% of the income of the local fishermen's associations (Gorelli et al., 2017). Presence of plastic debris in the digestive tract of *A. antennatus* has been reported sporadically while studying its diet (Cartes et al., 2008; Kapiris and Thessalou-Legaki, 2011) but no study has focused on MP ingestion and its effect for this species.

The current study aims to evaluate specifically the presence of MPs in the digestive tract of *Aristeus antennatus* from the western Mediterranean Sea and the possible origin of these pollutants. We hypothesize that the high economic activity developed around a densely populated and industrialized metropolitan area, such as Barcelona, acts as the main source of microplastics in the sea. Thus, the occurrence of plastics should vary along a gradient of distance to Barcelona city. In addition, the relationship between the presence of MPs and shrimp's diet or its effect on shrimp's health condition is explored for the first time.

2. Materials and methods

2.1. Sampling and data collection

Individuals of *Aristeus antennatus* were collected from different sites in the Balearic Basin (northwestern Mediterranean Sea). Fifteen samplings (hauls) were performed between 2008 and 2011, during different seasons (twelve of them during late spring and early summer) at depths ranging between 620 and 1870 m (Table 1). All samplings were carried out within the framework of the research projects BIOMARE and ANTROMARE (Spanish Ministry of Science and Innovation) on board of the research vessel García del Cid using a semi-balloon otter-trawl, OTSB-14. Sampling points were grouped into three categories according to their distance to Barcelona city, i.e. distance 1 (under 32.4 Nm of Barcelona), distance 2 (between 32.4 and 64.8 Nm of Barcelona) and distance 3 (over 64.8 Nm of Barcelona).

Specimens were immediately frozen on board and kept at −20 °C until further inspection. Once in the laboratory, shrimps were measured (cephalothorax length, CL, in mm; and total weight, TW, in g) and dissected. Presence of MP was recorded for each individual when fibers or fragments potentially made of plastic were clearly found embedded in the gut content (alimentary bolus). Moreover, MP fibers can occur as isolated fibers or tangle up into balls, in any case, the occurrence of isolated MP or MP balls was annotated separately. In addition, in a subsample of 29 individuals (see Table 1 for details); detailed analysis

Table 1
Sampling data for *Aristeus antennatus* included in the spatial analysis of the presence of microplastics in stomachs and analysis of biological condition and diet. Loc: distance-based classification into three categories, distance 1 (d1), distance 2 (d2) and distance 3 (d3); n = number of individuals analyzed for spatial occurrence of shrimps with microplastics in their stomachs; * individuals also included in the analysis of diet and biological condition.

Trawl	Date	Depth (m)	Latitude (deg, min, N)	Longitude (deg, min, E)	Loc	n
B805	26/02/2008	996.5	41° 09.32	2° 30.06	d1	6
A104	08/07/2010	1024	40° 58.69	2° 01.14	d1	27
A314	22/10/2011	1028	40° 57.99	2° 02.87	d1	9
A111	13/07/2010	1630	40° 56.40	2° 30.03	d1	4
A204-6	19/06/2011	638	40° 54.36	1° 39.22	d2	9*
A109	12/07/2010	1743.5	40° 38.39	2° 04.12	d2	10
A110	12/07/2010	1787	40° 30.81	2° 03.68	d2	9
A207-8	20/06/2011	626	40° 40.92	1° 26.44	d2	10*
A201-2	18/06/2011	643	40° 34.48	1° 26.47	d2	10*
A122	21/07/2010	1873.5	40° 23.30	2° 40.65	d2	10
A120	20/07/2010	1605	40° 08.53	2° 12.21	d3	8
A121	20/07/2010	1477	40° 05.37	2° 11.33	d3	9
A119	19/07/2010	1231.5	39° 55.16	2° 08.25	d3	10
A117	19/07/2010	1006	39° 52.39	2° 20.26	d3	10
A312	19/10/2011	1407	39° 45.53	1° 44.88	d3	7

of shrimp's condition and diet was performed. Hepatopancreas weight (HW) in g was recorded and shrimp condition was assessed by condition factor (as $K = (TW/CL^3) \times 100$) and hepatosomatic index (as $HSI = (HW/TW) \times 100$). Analysis of diet is further explained in Section 2.3.

An additional sampling was performed in 2017 in order to characterize shrimp's stomach morphology and MPs. Shrimps were collected from a commercial vessel off Barcelona at a depth of 974.5 m (41° 09.65′ N; 2° 18.40′ E). Eleven individuals were immediately fixed in 10% buffered formalin and later processed in order to characterize shrimp's stomach morphology and MPs found within (see Section 2.4 for details concerning microplastic characterization).

Measures were adopted while handling and processing the samples in order to prevent airborne contamination (Woodall et al., 2015). Work surfaces and dissection material were swiped cleaned with alcohol and revised under the stereomicroscope and nitrile gloves and 100% cotton lab coat were worn at all times.

2.2. Spatial occurrence of microplastics in stomachs

Occurrence of MP (isolated fibers or fragments, balls or both) was calculated for each sampling point as the number of individuals with MP in their stomachs divided by the number of individuals examined. Differences in MP occurrence between distance-based categories were tested for each possibility (i.e. isolated fibers, balls or both) using two-way ANOVA including depth and shrimp size (CL) as covariates, followed by Tukey's HSD post-hoc pairwise comparisons. Correlation between MP and ball occurrence was also explored by simple linear regression analysis (SLR). Statistical analyses were run using RStudio (Version 1.0.136).

Following dissection, stomachs from the eleven individuals sampled in 2017 were used to characterize stomach's morphology. Briefly, stomachs were cleaned in 10% KOH solution and stained with Alizarin-Red based on Castejón et al. (2015) protocols and close-up images were taken using an Olympus Tough TG-3 Stylus camera.

2.3. Detailed analysis of diet and biological condition

In a subsample of 29 individuals, after the examination of stomachs for the presence of MP and balls, all stomach content was weighed (CW) to the nearest 0.1 mg. Prey were identified to the lowest possible taxonomic level under a stereomicroscope and weighted. Since shrimps crush and cut prey in a number of small pieces, it was not often possible to obtain a direct weight for each prey item. Then, weight of prey consumed was estimated after the percentage volume occupied by each prey in stomach content using the subjective points method (Swynnerton and Worthington, 1940). This methodology has been successfully used in previous works that analyzed this shrimp's diet (Cartes, 1994; Cartes et al., 2008). The whole stomach content weight was thereby partitioned for each prey-type. Feeding intensity was measured through a fullness stomach index calculated as follows $F = CW/TW \times 100$, in wet weight. Shannon-Wiener index (H′) (Shannon and Weaver, 1948) and Eveness (J′) (Pielou, 1975) were used to calculate trophic (diet) diversity.

In addition, diet items were classified into four ecological categories depending on habitat and movement/swimming capacity of prey (endobenthos, epibenthos, hyperbenthos and plankton) and the following variables were calculated for each individual:

- Total biomass (TB) as the absolute biomass of all diet items for each ecological category.
- Relative biomass (RB) as the percentage of total biomass for each ecological category in relation to total gut content.
- Relative diversity (RD) as the percentage of taxonomic items present in each individual in relation to the total number of taxonomic items identified for the same category in all individuals.

Possible differences on shrimp's biological variables (CL and TW), condition indices (K and HSI), feeding intensity (F) and diet diversity (H′ and J′ indices) according to MP presence were tested by means of ANOVA after square-transformation of the data. Multivariate data on diet composition was also analyzed by Principal Coordinate Analysis (PCoA) performed on Bray-Curtis matrices derived from square-transformed data and followed by PERMANOVA analysis in order to explore differences related to MP presence. Permutation *p*-values were obtained under unrestricted permutation of raw data (9999 permutations). Similarity percentage analysis were carried out in order to explore which taxa contributed the most to similarity/dissimilarity between groups according to MP presence. Finally, we searched for possible trends in TB, RL and RD and ecological categories in relation to plastic presence at two levels: (1) by PCoA techniques coupled with PERMANOVA analysis, and (2) by ANOVA tests (or Wilcoxon Rank Sum tests if normality conditions were not accomplished after square root transformation of data). In the former, we defined as variables the combination of TB, RB and RD with ecological variables, and therefore carrying each analysis with four variables (e.g. total endobenthic biomass, total epibenthic biomass, total hyperbenthic biomass and total planktonic biomass for TB multivariate analysis). Principal Coordinate Analysis were carried out with Bray-Curtis similarity matrix and plotted in two dimensions. Furthermore, Pearson's correlation for each variable and PCoA axis was calculated. In the latter, we compared TB, RB and RD for each ecological category at a time using plastic presence as factor in order to determine which category was responsible for the differences detected in multivariate analysis. All pairwise comparison tests (ANOVA and Wilcoxon) were performed in RStudio (Version 1.0.136) and PCoA and PERMANOVA analysis were performed with PRIMER PERMANOVA+ 6 (Anderson et al., 2008).

2.4. Characterization of microplastics

Only MPs recovered from the dissection of shrimps sampled in 2017 for stomach's characterization purposes were analyzed. Following dissection, MPs were saved individually. When a ball of tangled up fibers was found, each fiber was carefully separated. All MPs were described in terms of aspect -color, maximum length and mean width (calculated on the basis of three random measures)-. Images and measures were taken with a Leica camera attached to a microscope using ProgRes® CapturePro 2.7 calibration software. The polymer composition was identified using Fourier transformed infrared (FTIR) spectroscopy technique. Organic debris was manually removed from items, which were rinsed and dried prior to analysis. Spectra were collected in transmission mode in 16 scans with a resolution of $1.92\,cm^{-1}$ using a Tensor 27 FTIR spectrometer (Bruker Optki GmbH, Ettlingen, Germany) equipped with a diamond attenuated total reflectance (ATR) unit. Resulting spectra was manipulated with The Unscrambler® X (Version 10.4) software in order to apply baseline corrections, normalize characteristic peaks and apply signal corrections in test samples. Then, each corrected spectrum was compared with reference spectra. Spectra from 11 common reference polymers were included in a self-generated library (acrylic, cellulose, elastane, nylon/polyamide, low-density and high-density polyethylene, polyethylene terephthalate (including samples used in packaging and textile –polyester–), polypropylene, polystyrene, polyurethane, and rayon/viscose). Similarity correlation indices between sample and reference spectrum were calculated for characteristic bands (from 1800 to $670\,cm^{-1}$ wavelengths) and values > 70% similarity were selected. Results were further checked by visual correlation of peaks and by using the KnowItAll® (Bio-Rad, USA) software in order to compare spectra with a broader database.

3. Results

3.1. Spatial occurrence of microplastics in stomachs

A total of 58 out of 148 (39.2%) individuals of *Aristeus antennatus* contained MPs inside their stomachs. The vast majority of items found were fibers that, in some cases, were tangled up in balls (Fig. 1). These fibers might be considered meso- or macroplastics according to their length (sometimes surpassing 5 mm) but are named microplastics based on their diameter (< 1 mm).

Microplastic balls were often found near the gastric mill during dissection. Macroscopic observation of stained stomachs revealed at the gastric mill the presence of numerous chitinous teeth and plates

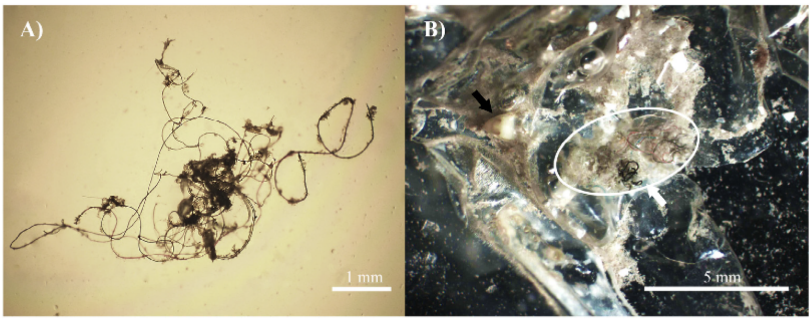


Fig. 1. Aggregation of microplastic fibers tangled up in balls, recovered from the stomach content of *Aristeus antennatus* individuals captured in the Balearic Basin, (northwestern Mediterranean Sea). A) Isolated ball made up of more than five fibers of different colors and diameter. B) Size of a ball (white circle and arrow) in relation with the size of the chitinous teeth of the stomach (black arrow).

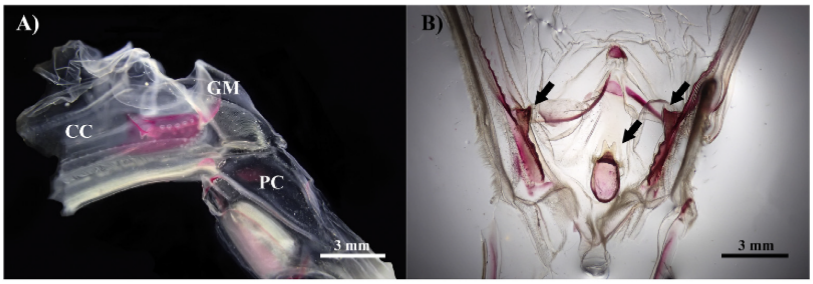


Fig. 2. Macroscopic view of the stomach of adult individuals of *Aristeus antennatus* stained with Alizarin Red (A–B; macroscopic view). A) Lateral view of the stomach. CC = cardiac chamber; GM = gastric mill; PC = pyloric chamber. B) Ventral view of the gastric mill opened up. Calcified structures (the chitinous teeth) appear stained in bright pink. Black arrows indicate median and lateral tooth. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

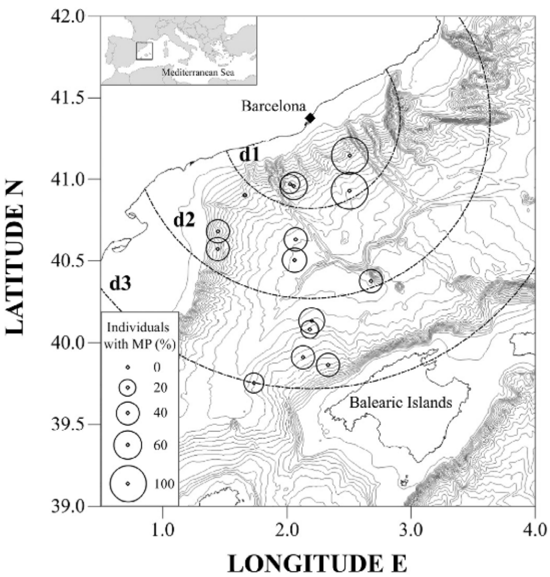


Fig. 3. Spatial occurrence of microplastics (MP) in stomachs of *Aristeus antennatus* captured in Northwestern Mediterranean Sea. The size of the circles represents the percentage of individuals with MPs inside their stomachs. Dotted lines divide the study area into three categories based on the distance to Barcelona city.

situated at the entrance of the hindgut. In particular, our observations confirmed the presence of two lateral and one median protruding tooth (Fig. 2), and several setae.

Percentage of MP occurrence (i.e. percentage of shrimps with MP inside their stomachs) varied for hauls between 0 and 100% (Fig. 3). The highest values were found for hauls B805 and A111, both relatively close to Barcelona city (d1), while no individuals containing MPs were found in A204–6 hauls (d2). There was a trend to higher MP occurrence in hauls performed close to Barcelona city, with marginal significant differences between d1 and d2-d3 ($F_{2,12} = 3.784$, $p = 0.0532$) (Table 2). The occurrence of MP balls ranged between 0 and 33.3% and no differences were found among distance-based categories. When only shrimps with MPs were taken into consideration, one out of four individuals had balls of MP fibers. A positive relationship between the occurrence of isolated fibers and the occurrence of balls was observed ($R^2 = 0.42$; $F_{1,13} = 11.03$; $p = 0.006$). In any case, no trends were observed neither for depth of capture, that ranged between 600 and 1800 m, nor shrimp's size (CL), with individuals ranging between 20.3 and 56.8 mm.

Table 2
Occurrence of microplastics (MP) in stomachs of *Aristeus antennatus* (mean and standard deviation) according to the distance to Barcelona city. Distance-based classification into three categories, distance 1 (d1; < 32.4 Nm), distance 2 (d2; 32.4 Nm) and distance 3 (d3; > 64.8 Nm). Sample size refers to the number of hauls (and individuals) included in that distance category (see Details in Table 1). Different superscript letters show marginally significant differences ($0.1 < p < 0.05$) in spatial assessment.

Distance to source	d1	d2	d3
Sample size	4 (46)	6 (58)	5 (44)
% MP (isolated fibers or fragments)	52.1 ± 23.7^a	23.9 ± 16.7^b	26.3 ± 9.5^b
% MP (fibers tangled up in balls)	18.3 ± 13.4^a	10.2 ± 12.7^a	9.9 ± 10.5^a
% Total MP	70.4 ± 36.3^a	34.1 ± 16.8^b	36.2 ± 10.9^b

3.2. Detailed analysis of diet and biological condition

All individuals (29) examined in this subsample were adults with lengths ranging between 31.5 and 37.6 mm (CL). MP presence was confirmed in eight stomachs (27.6%) and individuals were grouped according to presence/absence of MPs. No differences were found between these groups for shrimp biological variables (CL and TW), condition indices (K and HSI) or stomach fullness (F) ($p > 0.05$) (Table 3).

The diet of *A. antennatus* consisted of 33 items that belonged mainly to polychaetes, small crustaceans (e.g. decapods, amphipods or euphasiids), fish remains and mollusks (bivalves and gastropods). Each item was classified into the following ecological categories: plankton, hyperbenthos, epibenthos or endobenthos (Table 4).

Permutational multivariate analysis (PERMANOVA) of the diet composition showed marginal significant differences among those individuals with plastics and those without ($\text{Pseudo-}F_{(1,28)} = 1.5688$, $p_{(perm)} = 0.0678$; 9910 unique permutations). No differences were observed in diet diversity indexes (H′ and J′; $p > 0.05$). The similarity percentage analysis (SIMPER) showed that the taxa that contributed the most (between 5 and 10%, cumulative contribution 32.93%) to dissimilarity among MP presence groups were Alcyopidae, *Natatolana*

Table 3
Mean values and standard deviations of shrimp's biological variables and trophic indices. CL: cephalothorax length; TW: total weight; K: condition factor; HSI: hepatosomatic index; F: fullness index; MSR: Mean species richness; H′: Shannon's diversity index; J′: Pielou's eveness index.

	Absence of MP	Presence of MP
CL (mm)	33.90 ± 1.51	33.93 ± 0.71
TW (g)	14.18 ± 2.05	14.29 ± 1.58
K	0.036 ± 0.004	0.036 ± 0.003
HSI	6.88 ± 1.44	5.78 ± 1.91
F	0.68 ± 0.32	0.48 ± 0.25
MSR	7.52 ± 0.16	8.38 ± 2.77
H′	0.73 ± 0.16	0.75 ± 0.09
J′	1.42 ± 0.59	1.58 ± 0.37

Table 4
Classification of diet items identified into ecological categories according to their position in relation to the sea bottom. Total number of taxonomic items identified is stated along with detailed references of the items included in each category.

Ecological category	n° taxa	Description of taxonomic items included
Plankton	8	Siphonophores (*Chelophyes appendiculata*), polychaetes (Alcyopidae), euphasiids (*Meganyctiphanes norvegica* and *Nematoscelis megalops*), decapod crustaceans (Sergestidae and larvae), hyperiid amphipods and myctophid fish.
Hyperbenthos	4	Polychaetes (Polynoidae), (decapods (*Aristeus antennatus* and *Plesionika martia*)) and mysids (*Boreomysis arctica*).
Epibenthos	12	Gammarid amphipods (*Bruzelia typica*, *Harpinia* spp., *Rhachotropis caeca*, Lysianassidae and Oedicerotidae), isopods (*Eurydice* sp., *Natatolana borealis*), gastropods (Pteropoda: sedimented shells), caudofoveats, holothuroids and fish remains.
Endobenthos	9	Polychaetes (*Glycera* spp. and Eunicidae), mollusks (bivalves *Abra longicallus*, Taxodonta), decapods (*Calocaris macandreae*), cumaceans (*Leucon longirostris*), benthic ostracods, sipunculans and foraminiferans.

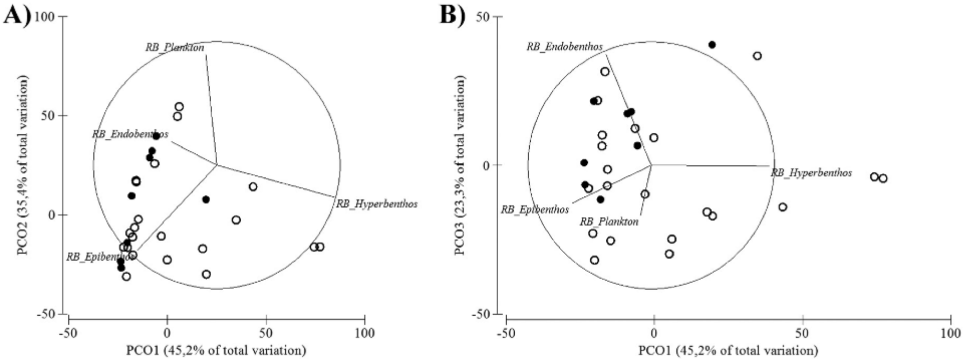


Fig. 4. Application of principal coordinates analysis (PCoA) to arrange shrimp individuals based on their diet composition according to relative biomass (RB) of each ecological category (plankton, hyperbenthos, epibenthos and endobenthos) using the combination of first and second components (A) and first and third components (B). Bray-Curtis similarity was used to calculate distances among all individuals. Lines represent the correlation (Pearson's correlation) between each variable and the PC axis. The outer circle represents a correlation = 1. Samples are represented as either filled points (individuals with microplastics) and un-filled points (individuals without microplastics).

borealis, *Calocaris macandreae*, *Rhachotropis caeca* and Lysianiassidae.

Multivariate analysis of the ecological composition of diet showed significant differences between MP presence groups only for relative diversity (Pseudo-$F_{1,28}$ = 3.8188; $p_{(perm)}$ = 0.0194; 9940 unique perm.). PCoA results coupled with Pearson's correlation showed a trend towards shrimps with MP having a higher contribution of endobenthos to diet while shrimps without plastics were more related with hyperbenthic (swimming benthos) diversity. Similar trends were observed for relative biomass (Fig.4).

These results were further supported by univariate analysis for each ecological category at a time. Relative biomass and relative diversity of endobenthos was higher in shrimps with MP in their stomachs in comparison with those without ($F_{1,27}$ = 6.164; p = 0.0195 and $F_{1,27}$ = 4.262; p = 0.0487, respectively) while contribution of hyperbenthos to diet in terms of total and relative biomass was higher in shrimps without MP (W = 121.5; p = 0.04785) (Fig. 5).

3.3. Characterization of microplastics

A total of 13 potential plastic items were visually sorted from the stomach content of the individuals used for stomach's morphology characterization. All items found, except for one film-like particle, were fibers. Fibers varied in size with length ranging between 1.9 and 26.7 mm (median 6.6 mm) and width ranging from 0.012 to 0.032. Fibers from five different colors (transparent, black, blue, green and red) were found, without a clear preference for color. Seven items were successfully analyzed with FTIR (53.8% hit-rate) and identified as three different polymer types: polyethylene terephthalate (polyester) (57.1%), nylon/polyamide (28.6%) and rayon/viscose (14.3%).

4. Discussion

This is the first assessment of MP presence in stomachs of a Mediterranean deep-sea decapod crustacean, in this case a species of high commercial interest such as *Aristeus antennatus*. In addition, MP occurrence along a spatial gradient from Barcelona metropolitan area in the NW Mediterranean Sea is analyzed. Moreover, the effect of MP occurrence in stomachs on the condition, trophic indices and diet composition of shrimps is explored.

Our results successfully demonstrate that MP commonly occur inside the stomachs of *A. antennatus* even in deep areas free of trawling activity (> 1000 m), ingestion during natural feeding being the most likely route of entry. Several studies have confirmed the occurrence of MP inside the digestive tract of other deep-dwelling species in the Mediterranean Sea, such as *Pagellus bogaraveo* (Anastasopoulou et al., 2013) or *Galeus melastomus* (Alomar and Deudero, 2017; Anastasopoulou et al., 2013). In fact, ingestion of anthropogenic debris, even though at low values of occurrence, has been confirmed in several species, including six teleosteans and three sharks, e.g. the own *Galeus melastomus*, retrieved from close or even the same samplings performed in this study (Cartes et al., 2016). In the case of *A. antennatus*, MP ingestion seems a rather common phenomenon as it was reported from nearly all sampling points. However, overall, fewer than half of the individuals examined had MPs inside their stomachs. This percentage falls far below the 83% of individuals affected in another crustacean decapod, the Norway lobster *Nephrops norvegicus*, in the Clyde Sea (Murray and Cowie, 2011) and would be on the same order as those reported for benthic invertebrates (48% across all species analyzed) (Courtene-Jones et al., 2017) or for demersal and pelagic fishes (about 40%) (Lusher et al., 2013). Comparisons between MP incidence studies is tricky given the great variety of methodologies followed and the

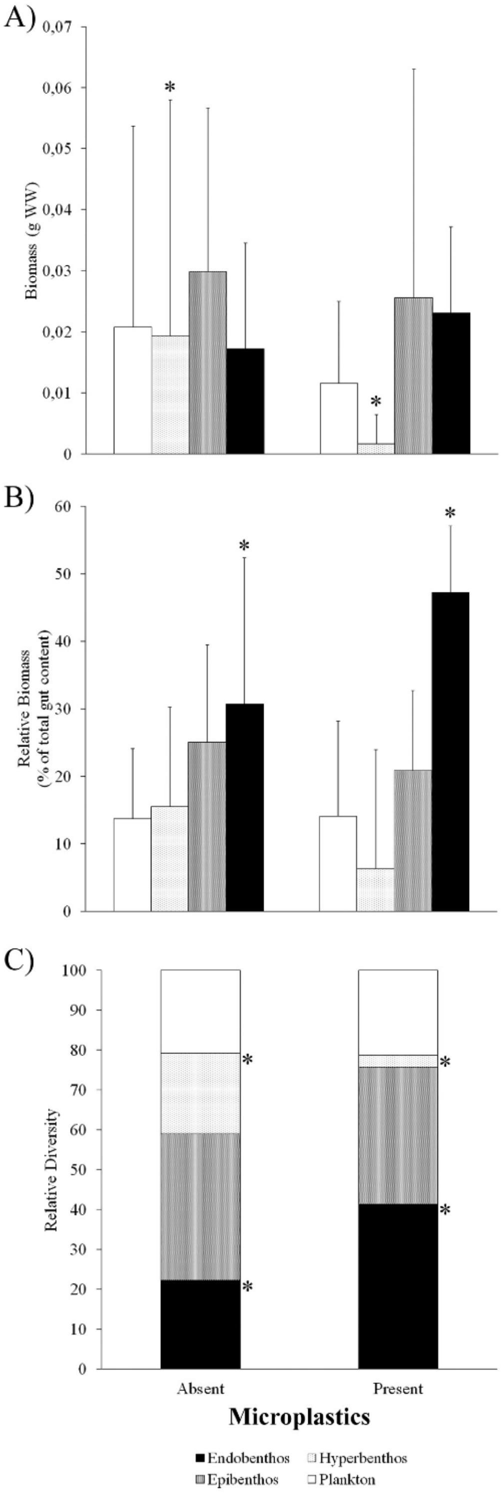


Fig. 5. Mean values and standard deviations for total biomass (A), relative biomass (B) and relative diversity (C) according to ecological category in which diet items were classified and grouped by the microplastic presence/absence factor. * Indicates significant differences ($p < 0.05$).

possible effect of airborne contamination (Hermsen et al., 2017). In our case, MPs found were always embedded in the alimentary bolus of shrimps and, the occurrence of balls (not a possible effect of airborne contamination) and isolated fibers were significantly correlated ($p < 0.001$), suggesting that isolated fibers, as happens with balls, were found in stomachs following ingestion. Finally, some fibers collected from stomachs presented, under the scanning microscope (SEM), attached remains that marked a Ca peak in elemental analysis (author's unpublished). This Ca peak is attributable to remains of the abundant calcified prey (e.g. foraminiferans, crustaceans or bivalves) consumed by *A. antennatus* (Cartes, 1994; Cartes et al., 2008).

Great values of MP ingestion in the vicinity of Barcelona metropolitan area give only partial support of our hypothesis that it acts as the main source of MP pollution, since there was not a clear decreasing trend in the outermost area. Closest areas, due to their proximity, might be highly influenced by land-based sources of MP pollution such as sewage. Current wastewater treatment plants (WTP) are neither efficient nor capable of removing the large amount of synthetic microfibers present in residual waters (Murphy et al., 2016). These fibers, that most likely come from domestic washing (a single garment can shed up to 1900 fibers every time it is washed), go through the WTP and ultimately reach the sea (Browne et al., 2011; Cesa et al., 2017; Woodall et al., 2014). On the other hand, presence of microplastic pollution in distant areas could be related to other non-foreseen land-based sources located in along the Catalan coast or in the Balearic Islands, to great dissemination rates of MP pollution from coastlines or to the contribution of marine-based sources, like commercial fisheries. In fact, deep-bottom trawling fisheries, that have traditionally targeted fishing grounds located near the port of Barcelona (Sardà et al., 1997), might contribute to MP pollution in both close and far areas. Bottom-trawlers can remobilize litter deposited on the seafloor and ease its movement to greater depths (Puig et al., 2012) while lost or damaged fishing gears and nets can act as potential microplastic sources (GESAMP, 2015). The composition of polymers identified from a small subsample of close-to-Barcelona individuals do not help elucidate which are the most important sources. For instance, polyamide (nylon) and polyethylene terephthalate (polyester) are widely used in both textile industry (land-based source) and fishing gears (marine-based source) (Wright et al., 2013b). Only in the case of rayon, land-based sources can be clearly pointed out. This man-made semi-synthetic material, also known as viscose or modal, is widely used in hygiene products, cleaning utensils and clothing (Lusher et al., 2013). Similar composition of polymers has been found in surveys conducted in the Atlantic and Mediterranean oceans (Kanhai et al., 2017; Lusher et al., 2013; Woodall et al., 2014) with similar potential sources being identified. Overall, there are still many gaps in the knowledge of plastic abundance and distribution as well as the local or regional importance of land- and marine-based sources, and correlations with densely populated or industrialized areas may not be as predictable or as simple because of complex transport mechanisms (Law and Thompson, 2014). In particular, the hydrodynamic conditions of areas like the Balearic Basin, with a dense system of canyons close to the shoreline and frequent dense water shelf cascading effects, might enhance the interception and transport of great amounts of plastic debris to the close deep areas (d1) (Tubau et al., 2015). Bathyal sediments in the Balearic Basin are mud and similar content of total organic matter (> 12% TOM) even at highest depths (to 2000 m, Cartes et al., 2002; Fanelli et al., 2013) may indicate high sediment deposition along all the slope. At greater depths, resuspension of deposited sediments by bottom currents (Puig et al., 2012) reported within the Western Mediterranean Deep Water (WMDW) mass could contribute to an homogeneous dispersion of microplastics over all the Balearic Basin (d2 = d3). At the same time, it should be taken into account that differences in diet between mainland and insular populations of shrimps (i.e. higher contribution of zooplankton in *A. antennatus* diet close to the Balearic Islands) or in relation to depth (Cartes, 1994; Cartes et al., 2008) might also play a key role in plastic ingestion and thus interfere in the correlation between MP ingestion and MP occurrence in the environment.

Aristeus antennatus has a great ability to root in mud and preys mostly on endobenthic and epibenthic invertebrates (Cartes, 1998, 1994). This close relationship with the substrate might favors a great exposure to MPs accumulated in the seabed (Van Cauwenberghe et al., 2015) that would be accidentally ingested during shrimp's normal feeding activity (Lusher et al., 2013). For example, some species of pelagic and demersal fishes such as *Sardina pilchardus* or *Platichthys flesus* with filter-feeding strategies have been observed to passively ingest MPs while filtering great volumes of water or mud, respectively, in search of prey items (Collard et al., 2017; Rummel et al., 2016). Murray and Cowie (2011) proposed a similar hypothesis in the case of the Norway lobster, *Nephrops norvegicus*, not being able to discriminate between plastic fibers and food and ingesting them by accident. As a crustacean with well-developed sight sense, it is also possible that *A. antennatus* actively preys upon MP fibers (as balls) because they can be similar in size to small polychaetes, an important diet prey of *A: antennatus* (Cartes, 1994; Cartes and Sardà, 1989). Resemblance between plastics and prey has been highlighted in the case of *Decapterus muroadsi* ingesting blue plastic particles resembling its blue copepod common prey or in *Scomber scombrus* eating red plastic fibers resembling juvenile Syngnathidae (Ory et al., 2017; Rummel et al., 2016), both rather visual predators. In these cases, selective does not prevent plastic ingestion – quite the contrary – plastic items might be ingested as well as they are not differentiated from natural prey (Wright et al., 2013b). Another possible route of entry might be trophic transfer, as proposed by Eriksson and Burton (2003) for the plastic particles found in fur seals, probably acquired through their fish prey. In this same way, observation in some stomachs of MP fibers deeply embedded in undigested tissues might indicate that our deep-sea shrimps could ingest MP fibers attached to detritic tubes of polychaetes (also often found in *A. antennatus* stomachs; Cartes, 1994). Trophic transfer has only been confirmed for micro-sized plastics (0.5 μm) in experimental studies conducted with *Mytilus edulis* and *Carcinus maenas* (Farrell and Nelson, 2013). No studies of MP ingestion have targeted prey species of *A. antennatus* in the Mediterranean Sea to date, but it has been reported for several invertebrate species in other areas (Browne et al., 2008; Courtene-Jones et al., 2017; Graham and Thompson, 2009; Wright et al., 2013a) and therefore it seems likely to occur also in prey species (e.g. polychaetes) of *A. antennatus*.

The vast majority of MP found were fibers, with the nearly absence of other shapes. Given that both fibers and fragments are expected to be present in the environment, as reported in sediments near the Balearic Islands (Alomar et al., 2016), this bias might more likely reflect that fibers are more easily ingested and retained inside shrimp's stomach (Welden and Cowie, 2016a). Furthermore, as mentioned before, isolated MP fibers might be attached to prey species of *A. antennatus* such as polychaetes and active feeding might play a role in increasing their ingestion. Once inside the stomach, MP fibers may be retained due to stomach's morphology and the formation of balls (Welden and Cowie, 2016b), probably on account of the numerous protruding and serrated chitinous pieces observed. The complexity of the digestive system of *A. antennatus*, here depicted for the first time, may hinder the transit of fibers through the digestive system, as they would have to be orientated straight to end to pass through the gastric mill (Murray and Cowie, 2011). In fact, loss of plastics may only occur in rare occasions, in larger individuals that may have proportionally more space in the gastric mill for plastics to pass through, or via moulting (Welden and Cowie, 2016b). During moult, the entire foregut lining, including the gastric mill, is shed and, therefore, it might act as a primary route for plastic loss. Moult in *A. antennatus* occurs, depending on the reproductive cycle, every few months (at least 2–3) (Demestre, 1995). Moreover, during the digestion process the gastric mill grinds the food particles (McGaw and Curtis, 2013; Patwardhan, 1935) yet, when facing strands of plastic material, the cut-and-grinding movements performed, in addition to the morphology of the teeth itself, might tangle fibers up into balls rather than breaking them down. Welden and Cowie (2016a) confirmed in experimental studies that plastic balls were formed in *N. norvegicus* fed on single plastic strands (Welden and Cowie, 2016a). The formation of balls increases overall size of plastic debris, hindering their excretion and increasing time of residence, which ultimately increases the likelihood of negative effects upon shrimp's condition (Welden and Cowie, 2016a).

Dietary composition of *Aristeus antennatus* matched with that reported in precedent studies (Cartes, 1994). Differences observed in diet composition might be related to natural differences on feeding habits of *A. antennatus* individuals rather than a change on diet behavior because of MP ingestion. Also, differences according to size or sex can be discarded as the individuals selected were from similar trawls and biological characteristics. Individuals with a higher preference for endobenthic organisms would have been more exposed to MP accumulated in the upper (surface and subsurface) layers of sediment and would have incorporated more fibers through their normal feeding activity. This is supported by the composition of the diet in terms of taxa, with exclusive presence of planktonic and hyperbenthic preys in individuals without MP fibers (e.g. *Chelophyes appendiculata* or *Boreomysis arctica*); and biomass, with higher contributions of endobenthic preys such as *Calocaris macandreae* in shrimps with MP fibers. These results are in accordance with the work of Peters et al. (2017) who also found a close relation between the ingestion of benthic items (sand and mollusks) and the presence of microplastics in the fish *Orthopristis chrysoptera*.

Presence of MP has been related to several negative effects including starvation and intestinal blockages that can lead to the depletion of energy stores and reduced body condition due to their low or null nutritional value (Welden and Cowie, 2016a; Wright et al., 2013b). These effects seem particularly plausible in the case of MP balls getting stuck and interfering totally or partially in the gastric mill's functioning of *A. antennatus*, especially in view of the size observed in some cases (> 5 mm). *Aristeus antennatus* has a relatively high food consumption (daily ration) with a rather fast evacuation rate of food ($R = 0.179\,h^{-1}$; (Maynou and Cartes, 1997), i.e. ca. 90% of food can be digested in ca. 5 h. In parallel, it has a relatively long moult time above indicated. Therefore, balls could persist enough in guts to generate a negative effect on shrimps' condition. Despite of that, no differences in trophic indices, including stomach fullness, or ultimately shrimp's condition were identified.

5. Conclusions

Microplastics, especially fibers, must be ingested during normal feeding activity of *A. antennatus* and possibly retained inside the foregut for long periods. Even though no clear effects upon condition or diet were observed, the great knowledge about diet, reproduction, morphology and spatial dynamics of this ecologically and commercially key species offers a great opportunity to assess how marine debris interferes with this species and even use it with monitoring purposes for deep-sea pollution. There are still many questions that remain unsolved and more research is needed in order to fill the gaps in the knowledge of microplastic distribution in the deep sea of the NW Mediterranean Sea, the routes and drivers that influence their ingestion in decapod crustaceans and, last but not least, what is their impact on feeding and health condition of deep-water species like *Aristeus antennatus*.

Acknowledgements

We are grateful to Dr. Santiago Maspoch for his assistance on the identification of plastic polymers by FTIR and to Bio-Rad Laboratories, Inc. for providing a free academic trial of its software KnowItAll. This study was supported by the Spanish Ministry of Science and Innovation (MICINN) projects "BIOMARE" (CTM2006-13508-C02-01MAR) and "ANTROMARE" (CTM2009-12214-C02-01/02). Carreras-Colom benefits from an FPU Ph.D. student grant from the MICINN (FPU16/03430).

References

Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark *Galeus melastomus* Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ. Pollut. 223, 223–229. http://dx.doi.org/10.1016/j.envpol.2017.01.015.

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1–10. http://dx.doi.org/10.1016/j.marenvres.2016.01.005.

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep-Sea Res. I Oceanogr. Res. Pap. 74, 11–13. http://dx.doi.org/10.1016/j.dsr.2012.12.008.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. Plymouth.

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. http://dx.doi.org/10.1016/j.marpolbul.2016.06.026.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastictranslocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42, 5026–5031. http://dx.doi.org/10.1021/es800249a. (CCC: $40.75).

Browne, M.A., Crump, P., Niven, S.J., Teuten, E.L., Tonkin, A., Galloway, T., Thompson, R.C., 2011. Accumulations of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 9175–9179. http://dx.doi.org/10.1021/es201811s.

Browne, M.A., Underwood, A.J., Chapman, M.G., Williams, R., Thompson, R.C., van Franeker, J.A., 2015. Linking effects of anthropogenic debris to ecological impacts. Proc. R. Soc. B Biol. Sci. 282http://dx.doi.org/10.1098/rspb.2014.2929. (20142929–20142929).

Cartes, J.E., 1994. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea (western Mediterranean). Mar. Biol. 120, 639–648. http://dx.doi.org/10.1007/BF00350085.

Cartes, J.E., 1998. Dynamics of the bathyal Benthic Boundary Layer in the northwestern Mediterranean: depth and temporal variations in macrofaunal-megafaunal communities and their possible connections within deep-sea trophic webs. Prog. Oceanogr. 41, 111–139. http://dx.doi.org/10.1016/S0079-6611(98)00018-4.

Cartes, J.E., Sardà, F., 1989. Feeding ecology of the deep-water aristeid crustacean *Aristeus antennatus*. Mar. Ecol. Prog. Ser. 54, 229–238. http://dx.doi.org/10.3354/meps054229.

Cartes, J.E., Sardà, F., 1993. Zonation of deep-sea decapod fauna in the Catalan Sea (Western Mediterranean). Mar. Ecol. Prog. Ser. 94, 27–34. http://dx.doi.org/10.3354/meps094027.

Cartes, J.E., Grémare, A., Maynou, F., Villora-Moreno, S., Dinet, A., 2002. Bathymetric changes in the distributions of particulate organic matter and associated fauna along a deep-sea transect down the catalan sea slope (Northwestern Mediterranean). Prog. Oceanogr. 53, 29–56. http://dx.doi.org/10.1016/S0079-6611(02)00023-X.

Cartes, J.E., Papiol, V., Guijarro, B., 2008. The feeding and diet of the deep-sea shrimp *Aristeus antennatus* off the Balearic Islands (Western Mediterranean): influence of environmental factors and relationship with the biological cycle. Prog. Oceanogr. 79, 37–54. http://dx.doi.org/10.1016/j.pocean.2008.07.003.

Cartes, J.E., Soler-Membrives, A., Stefanescu, C., Lombarte, A., Carrassón, M., 2016. Contributions of allochthonous inputs of food to the diets of benthopelagic fish over the northwest Mediterranean slope (to 2300 m). Deep-Sea Res. I Oceanogr. Res. Pap. 109, 123–136. http://dx.doi.org/10.1016/j.dsr.2015.11.001.

Cartes, J.E., López-Pérez, C., Carbonell, A., 2017. Condition and recruitment of *Aristeus antennatus* at great depths (to 2,300 m) in the Mediterranean: relationship with environmental factors. Fish. Oceanogr. 1–13. http://dx.doi.org/10.1111/fog.12237.

Castejón, D., Rotllant, G., Ribes, E., Durfort, M., Guerao, G., 2015. Foregut morphology and ontogeny of the spider crab *Maja brachydactyla* (Brachyura, Majoidea, Majidae). J. Morphol. http://dx.doi.org/10.1002/jmor.20404.

Cesa, F.S., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. Sci. Total Environ. 598, 1116–1129. http://dx.doi.org/10.1016/j.scitotenv.2017.04.172.

Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62, 2199–2204. http://dx.doi.org/10.1016/j.marpolbul.2011.06.030.

Collard, F., Gilbert, B., Eppe, G., Roos, L., Compère, P., Das, K., Parmentier, E., 2017. Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles. Mar. Pollut. Bull. 116, 182–191. http://dx.doi.org/10.1016/j.marpolbul.2016.12.067.

Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O.M., Narayanaswamy, B.E., 2017. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environ. Pollut. 231, 271–280. http://dx.doi.org/10.1016/j.envpol.2017.08.026.

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.Á., Irigoien, X., Duarte, C.M., 2015. Plastic accumulation in the Mediterranean Sea. PLoS One 10, 1–12. http://dx.doi.org/10.1371/journal.pone.0121762.

Demestre, M., 1995. Moult activity-related spawning success in the Mediterranean deep-water shrimp *Aristeus antennatus* (Decapoda: Dendrobranchiata). Mar. Ecol. Prog. Ser. 127, 57–64. http://dx.doi.org/10.3354/meps127057.

Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., Vethaak, A.D., 2015. Microplastic contamination in brown shrimp (Crangon crangon, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Mar. Pollut. Bull. 98, 179–187. http://dx.doi.org/10.1016/j.marpolbul.2015.06.051.

D'Onghia, G., Capezzuto, F., Mytilineou, C., Maiorano, P., Kapiris, K., Carlucci, R., Sion, L., Tursi, A., 2005. Comparison of the population structure and dynamics of *Aristeus antennatus* (Risso, 1816) between exploited and unexploited areas in the Mediterranean Sea. Fish. Res. 76, 22–38. http://dx.doi.org/10.1016/j.fishres.2005.05.007.

D'Onghia, G., Maiorano, P., Capezzuto, F., Carlucci, R., Battista, D., Giove, A., Sion, L., Tursi, A., 2009. Further evidences of deep-sea recruitment of *Aristeus antennatus* (Crustacea: Decapoda) and its role in the population renewal on the exploited bottoms of the Mediterranean. Fish. Res. 95, 236–245. http://dx.doi.org/10.1016/j.fishres.2008.09.025.

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9, 1–15. http://dx.doi.org/10.1371/journal.pone.0111913.

Eriksson, C., Burton, H., 2003. Origins and biological accumulation of small plastic particles in Fur Seals from Macquarie Island. AMBIO J. Hum. Environ. 32, 380. http://dx.doi.org/10.1639/0044-7447(2003)032[0380:OABAOS]2.0.CO;2.

Fanelli, E., Cartes, J.E., Papiol, V., López-Pérez, C., 2013. Environmental drivers of megafaunal assemblage composition and biomass distribution over mainland and insular slopes of the Balearic Basin (Western Mediterranean). Deep-Sea Res. I Oceanogr. Res. Pap. 78, 79–94. http://dx.doi.org/10.1016/j.dsr.2013.04.009.

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. http://dx.doi.org/10.1016/j.envpol.2013.01.046.

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. (IMO, FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP). In: Kershaw, P.J. (Ed.), Rep. Stud. GESAMP No. 90, (96 pp).

Gorelli, G., Company, J.B., Bahamón, N., Sardà, F., 2017. Improving codend selectivity in the fishery of the deep-sea red shrimp Aristeus antennatus in the northwestern Mediterranean Sea. Sci. Mar. 81, 381. http://dx.doi.org/10.3989/scimar.04575.25A.

Graham, E.R., Thompson, J.T., 2009. Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J. Exp. Mar. Biol. Ecol. 368, 22–29. http://dx.doi.org/10.1016/j.jembe.2008.09.007.

Hermsen, E., Pompe, R., Besseling, E., Koelmans, A.A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Mar. Pollut. Bull. 122, 253–258. http://dx.doi.org/10.1016/j.marpolbul.2017.06.051.

Kanhai, L.D.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Mar. Pollut. Bull. 115, 307–314. http://dx.doi.org/10.1016/j.marpolbul.2016.12.025.

Kapiris, K., Thessalou-Legaki, M., 2011. Feeding ecology of the deep-water blue-red shrimp *Aristeus antennatus* (Decapoda: Aristeidae) in the Greek Ionian Sea (E. Mediterranean). J. Sea Res. 65, 151–160. http://dx.doi.org/10.1016/j.seares.2010.09.005.

Law, K., Thompson, R.C., 2014. Microplastics in the seas - concern is rising about widespread contamination of the marine environment by microplastics. Science 345, 144–145. http://dx.doi.org/10.1002/2014EF000240/polymer. (80-.).

Lebreton, L.C.M., Greer, S.D., Borrero, J.C., 2012. Numerical modelling of floating debris in the world's oceans. Mar. Pollut. Bull. 64, 653–661. http://dx.doi.org/10.1016/j.marpolbul.2011.10.027.

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. http://dx.doi.org/10.1016/j.marpolbul.2012.11.028.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. http://dx.doi.org/10.1039/C6AY02415G.

Maynou, F., Cartes, J.E., 1997. Field estimation of daily ration in deep-sea shrimp *Aristeus antennatus* (Crustacea:Decapoda) in the western Mediterranean. Mar. Ecol. Prog. Ser. 153, 191–196. http://dx.doi.org/10.3354/meps153191.

McGaw, I.J., Curtis, D.L., 2013. A review of gastric processing in decapod crustaceans. J. Comp. Physiol. B 183, 443–465. http://dx.doi.org/10.1007/s00360-012-0730-3.

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50, 5800–5808. http://dx.doi.org/10.1021/acs.est.5b05416.

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. http://dx.doi.org/10.1016/j.marpolbul.2011.03.032.

Nadal, M.A., Alomar, C., Deudero, S., 2016. High levels of microplastic ingestion by the semipelagic fish bogue *Boops boops* (L.) around the Balearic Islands. Environ. Pollut. 214, 517–523. http://dx.doi.org/10.1016/j.envpol.2016.04.054.

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437. http://dx.doi.org/10.1016/j.scitotenv.2017.01.175.
Patwardhan, S.S., 1935. On the structure and mechanism of the gastric mill in Decapoda. III structure of the gastric mill in Anomura. Proc. Indiana Acad. Sci. 1, 359–375. http://dx.doi.org/10.1007/BF03050865.
Peters, C.A., Thomas, P.A., Rieper, K.B., Bratton, S.P., 2017. Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. Mar. Pollut. Bull. 124, 82–88. http://dx.doi.org/10.1016/j.marpolbul.2017.06.080.
Pielou, E.C., 1975. Ecological Diversity. John Wiley & Sons, New York.
Puig, P., Canals, M., Company, J.B., Martín, J., Amblas, D., Lastras, G., Palanques, A., Calafat, A.M., 2012. Ploughing the deep sea floor. Nature 489, 286–289. http://dx.doi.org/10.1038/nature11410.
Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95, 358–361. http://dx.doi.org/10.1016/j.marpolbul.2015.04.048.
Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. Mar. Pollut. Bull. 102, 134–141. http://dx.doi.org/10.1016/j.marpolbul.2015.11.043.
Ryan, P.G., Moore, C.J., van Franeker, J.A., Moloney, C.L., 2009. Monitoring the abundance of plastic debris in the marine environment. Philos. Trans. R. Soc. B 364, 1999–2012. http://dx.doi.org/10.1098/rstb.2008.0207.
Sardà, F., Maynou, F., Talló, L., 1997. Seasonal and spatial mobility patterns of rose shrimp *Aristeus antennatus* in the Western Mediterranean: results of a long-term study. Mar. Ecol. Prog. Ser. 159, 133–141. http://dx.doi.org/10.3354/meps159133.
Sardà, F., D'Onghia, G., Politou, C.Y., Company, J.B., Maiorano, P., Kapiris, K., 2004. Deep-sea distribution, biological and ecological aspects of Aristeus antennatus (Risso, 1816) in the western and central Mediterranean Sea. Sci. Mar. 68, 117–127. http://dx.doi.org/10.3989/scimar.2004.68s3117.
Shannon, C.E., Weaver, W., 1948. The mathematical theory of communication. MD Comput. Comput. Med. Pract. http://dx.doi.org/10.1145/584091.584093.
Swynnerton, G.H., Worthington, E.B., 1940. Note on the food of fish in Haweswater (Westmorland). J. Anim. Ecol. 9, 183–187.
Taylor, M.L., Gwinnett, C., Robinson, L.F., Woodall, L.C., 2016. Plastic microfibre ingestion by deep-sea organisms. Nat. Publ. Group 1–9. http://dx.doi.org/10.1038/srep33997.
Tubau, X., Canals, M., Lastras, G., Rayo, X., Rivera, J., Amblas, D., 2015. Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: the role of hydrodynamic processes. Prog. Oceanogr. 134, 379–403. http://dx.doi.org/10.1016/j.pocean.2015.03.013.
Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015. Microplastics in sediments: a review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. http://dx.doi.org/10.1016/j.marenvres.2015.06.007.
Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., Hughes, K.A., 2017. Microplastics in the Antarctic marine system: an emerging area of research. Sci. Total Environ. 598, 220–227. http://dx.doi.org/10.1016/j.scitotenv.2017.03.283.
Welden, N.A.C., Cowie, P.R., 2016a. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ. Pollut. 218, 895–900. http://dx.doi.org/10.1016/j.envpol.2016.08.020.
Welden, N.A.C., Cowie, P.R., 2016b. Environment and gut morphology influence microplastic retention in langoustine, *Nephrops norvegicus*. Environ. Pollut. 214, 859–865. http://dx.doi.org/10.1016/j.envpol.2016.03.067.
Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1http://dx.doi.org/10.1098/rsos.140317. (140317–140317).
Woodall, L.C., Gwinnett, C., Packer, M., Thompson, R.C., Robinson, L.F., Paterson, G.L.J., 2015. Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. Mar. Pollut. Bull. 95, 40–46. http://dx.doi.org/10.1016/j.marpolbul.2015.04.044.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013a. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. http://dx.doi.org/10.1016/j.cub.2013.10.068.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013b. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. http://dx.doi.org/10.1016/j.envpol.2013.02.031.

CHAPTER 4 A closer look at anthropogenic fiber ingestion in *Aristeus antennatus* in the NW Mediterranean Sea: Differences among years and locations and impact on health condition

Environmental Pollution 263 (2020) 114567

Contents lists available at ScienceDirect

Environmental Pollution

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

A closer look at anthropogenic fiber ingestion in *Aristeus antennatus* in the NW Mediterranean Sea: Differences among years and locations and impact on health condition☆

Ester Carreras-Colom [a], María Constenla [a], Anna Soler-Membrives [a], Joan E. Cartes [b], Mireia Baeza [c], Maite Carrassón [a, *]

[a] Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Barcelona, Spain
[b] Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta 37-49, 08003, Barcelona, Spain
[c] Departament de Química, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Barcelona, Spain

ARTICLE INFO

Article history:
Received 27 September 2019
Received in revised form
22 March 2020
Accepted 7 April 2020
Available online 15 April 2020

Keywords:
Fiber ingestion
Aristeus antennatus
Mediterranean sea
Plastic pollution
Health condition

ABSTRACT

Marine litter is one of the most concerning threats for marine wildlife especially regarding plastics and their micro-sized forms, widely known as microplastics. The present study evaluates mesoscale spatial (230 km, Catalan coast) and temporal (2007 vs 2017–2018, Barcelona area) differences on the ingestion of anthropogenic fibers in the deep-sea shrimp *Aristeus antennatus* in the NW Mediterranean Sea and its relation with shrimp's health condition. Synthetic fibers with lengths ranging between 0.16 and 37.9 mm were found in both stomach (where sometimes they were tangled up in balls) and intestine contents. The percentage of fiber occurrence was >65% at each sampling point. Tangled balls of fibers observed in stomach contents exhibited a wide range of sizes (up to a diameter of 1 cm) and were usually composed of fibers of different polymers, sizes and colours. Differences between locations (2018) were found, with greater fiber loads towards the south during spring and a great variability in summer, as shrimps caught off Barcelona showed a nearly thirty-times higher fiber load compared to shrimps from other localities. Highest concentrations were more likely to be related to major sources of fibers and currents in the area. Fiber load in shrimps from 2007 was comparable to that of shrimps captured in 2017 and 2018 (spring) yet a shift in the proportion of acrylic and polyester polymers was detected. No consistent effect on shrimp's health condition was found, with only a significant negative correlation found between gonadosomatic index and fibers for those shrimps with the highest values of fiber load (caught off Barcelona, summer 2018). Our findings contribute to the knowledge on plastic pollution for the NW Mediterranean Sea and highlight the potential use of this species as a sentinel species for plastic fiber contamination.

1. Introduction

Marine debris in particular plastic as the most common litter type has been identified as one of the major threats for marine ecosystems (UNEP, 2009). Jambeck et al. (2015) estimated that between 4.8 and 12.7 million metric tons (Mt) were entering the ocean in 2010 and more recently, Geyer et al. (2017) highlighted that if current production and waste management trends were to continue, up to 12,000 Mt could end up in the environment by 2050. The first synthetic plastic produced, Bakelite, only dates back to the 1950s, which means that plastic production and pollution have had unprecedented growth. Microplastics (plastic items <1 mm, Hartmann et al., 2019) have already been found in aquatic environments all around the world, from remote areas as the Antarctic system (Waller et al., 2017) or the Maldives Islands (Imhof et al., 2017) to coastal shallow areas close to urbanization (Alomar et al., 2016). Likewise, microplastic ingestion seems a widespread phenomenon and to date over 220 different marine species have been found to consume microplastics in the environment (Lusher et al., 2017).

The ubiquity of microplastics has raised awareness in all communities leading to an exponential increase in the number of

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.
* Corresponding author.
E-mail address: maite.carrasson@uab.es (M. Carrassón).

https://doi.org/10.1016/j.envpol.2020.114567
0269-7491/© 2020 Elsevier Ltd. All rights reserved.

studies focusing on this topic during the last decades (Lusher et al., 2017). However, the lack of standardized methods has led to hardly comparable results with even some inconsistencies in terminology (Hartmann et al., 2019). Many experimental studies have also attempted to assess the potential impact of microplastics on the health of organisms but consistent results are yet to be found since both negative and neutral responses on feeding, growth, reproduction and survival have been observed (Foley et al., 2018). Moreover, other fibers with anthropogenic origin such as rayon/viscose, essentially made of regenerated cellulose, or dyed cotton fibers, even though being made of naturally occurring polymers (e.g. cellulose) may also raise environmental concerns like their synthetic (i.e. plastic) counterparts (Ladewig et al., 2015). In order to allow for a correct assessment of hazards and risks posed by microplastics (and other anthropogenic particles), both experimental and field studies are needed. Even though we now have a great list of organisms for which plastic ingestion has been reported, studies focusing on understanding trends on release, transport and fate or on the factors related to microplastic ingestion are still scarce (Beer et al., 2018). Not to mention the importance of assessing whether plastic ingestion is increasing over time following the trends on global plastic production in order to better assess and forecast their potential impact (Beer et al., 2018). So far, only a couple of studies have addressed long-term trends in plastic ingestion in the Baltic and Atlantic Sea (Beer et al., 2018; Courtene-Jones et al., 2019).

The Mediterranean Sea has been described as one of the areas that is most polluted by plastics worldwide (García-Rivera et al., 2018; UNEP/MAP, 2015). High values of microplastic occurrence might be linked to the intense anthropogenic activity alongside the hydrodynamics of an enclosed basin. The great industrial activity and highly developed tourism, together with important fisheries and densely populated coastal segments might account for a great input of marine litter (Eriksen et al., 2014; Ramirez-Llodra et al., 2013). In the NW Mediterranean area, microplastic presence has been reported in surface waters (de Haan et al., 2019; Ruiz-Orejón et al., 2016; Schmidt et al., 2018), beaches (Constant et al., 2019), sediments (Sanchez-Vidal et al., 2018; Woodall et al., 2014) and several organisms, mainly coastal fish (Alomar and Deudero, 2017; Bellas et al., 2016; Collignon et al., 2012; Compa et al., 2018) but also in the deep-sea shrimp *Aristeus antennatus* (Carreras-Colom et al., 2018). In the latter, microplastic fibers might be retained for longer times compared to other organisms due to the presence of the gastric mill, a common food-grinding structure in decapods, which could hinder passage to the intestine (Watts et al., 2015; Welden and Cowie, 2016a, 2016b). Its action is also thought to favour the formation of tangled up balls of fibers with the corresponding increase in size and posing a major threat for health (Murray and Cowie, 2011).

Aristeus antennatus is an economically and ecologically important species in the deep-sea ecosystem whose biology, including reproduction, diet, population dynamics and most recently, microplastic ingestion, have been studied (Carbonell et al., 2006; Carreras-Colom et al., 2018; Cartes, 1994; Cartes et al., 2018; Demestre, 1995). After a first analysis focused on describing microplastic occurrence in stomachs from *A. antennatus* collected in 2010–2011 (Carreras-Colom et al., 2018), the analysis of individuals from 2007 from a previous project (BIOMARE) together with new material obtained in 2017–2018, pose a perfect opportunity to test whether the increase in plastic production is somehow reflected in the level of plastic ingestion in *A. antennatus* in these two time-points. This study analyses the occurrence, size and composition of anthropogenic fibers in the digestive tract of red shrimp (*Aristeus antennatus*) individuals from different years and locations along the Catalan coast (NW Mediterranean Sea). The main aims are: (1) to assess current (2018) spatial trends in fiber ingestion along the Catalan slope at a mid-scale distance (*ca.* 230 km), (2) to determine whether current levels of fiber ingestion (2017–2018) differ from those of 10 years ago in shrimps from an area with high anthropogenic pressure (off Barcelona), (3) to relate fiber ingestion to anthropogenic, environmental and biological factors, and (4) to assess the potential impact of fibers on shrimp's health condition.

2. Materials and methods

2.1. Study area and data collection

Shrimps were collected from three sites along the continental slope of the Catalan coast (NW Mediterranean Sea) from north to south: off Costa Brava, off Barcelona, and off the Ebro Delta. The sampling locations were selected in order to represent areas of different characteristics and levels of anthropogenic impact. The Costa Brava area, selected as the allegedly less impacted area given its less industrial activity and its location, the northern-most location in an area where southwards currents dominate. It is also characterized by the greatest seasonal shift in human population density (increase of about 19% in summer compared to spring) and is under the influence of the Tordera River (54 km length; 9.0 $hm^3\ y^{-1}$). The Barcelona area, suspected as the most impacted area given the previously reported values of fiber ingestion there (Carreras-Colom et al., 2018), is close to the largest and most dense urban and industrial areas along the shore and is subject to the influence of the Besòs (58 km length, 98.6 $hm^3\ y^{-1}$) and Llobregat (173 km length, 302.3 $hm^3\ y^{-1}$) rivers. The area off the Ebro Delta, whilst near a less densely populated area, is under the influence of the Ebro River's discharge (9281.0 $hm^3\ y^{-1}$), which is strongly affected by human activities, especially agriculture but also industry along its catchment area (85,569 km^2).

Sampling was performed in spring and summer of 2007 (in Barcelona), 2017 (in Barcelona and only spring) and 2018 (all locations and both seasons) within the framework of the research projects BIOMARE (Spanish Ministry of Science and Innovation) and SOMPESCA (Department of Agriculture, Livestock, Fisheries and Food, Catalonia, Spain) on board of commercial fishing vessels operating at depths ranging between 396 and 791 m (see Table 1 for details). All individuals ($n = 201$, Table 1) were immediately fixed in Davidson's AFA for 48–56 h and then preserved in ethanol 70%. Dissection was performed in a safety laminar flow cabinet and the digestive system (stomach and intestine) was removed and screened for the presence of anthropogenic particles using a

Table 1
Sampling data for *Aristeus antennatus*. Loc: locality, BC^B: off Barcelona city just in front of the Besòs River, BC^L: off Barcelona city just south of the Llobregat River, CB: off costa Brava, ED: off Ebro Delta. Season, sp: spring; su: summer. *n*: number of individuals; CL: cephalothorax length.

Code	Coordinates	Loc	Season	Year	Depth	*n*	CL (mm)
B2B	41.15 N; 2.40 E	BC^B	sp	2007	790	16	35.0–47.4
B3B	41.15 N; 2.40 E	BC^B	su	2007	790	31	22.9–46.6
B3V	41.07N; 2.22 E	BC^L	su	2007	791	20	22.5–44.7
P0B	41.18 N; 2.39 E	BC^B	sp	2017	785	25	33.8–42.5
P1G	41.39 N; 3.25 E	CB	sp	2018	641	22	26.8–31.2
P1V	41.04 N; 2.05 E	BC^L	sp	2018	759	23	26.8–35.6
P1D	40.13 N; 1.23 E	ED	sp	2018	551	9	33.4–37.3
P2G	41.47 N; 2.81 E	CB	su	2018	396	20	25.9–31.9
P2V	41.11 N; 1.94 E	BC^L	su	2018	572	18	31.1–38.3
P2D	40.33 N; 1.32 E	ED	su	2018	425	17	33.1–40.7

stereomicroscope. For each individual, total body weight (TeW; eviscerated – without stomach, gonad and hepatopancreas – and without appendices), cephalothorax length (CL), and hepatopancreas (digestive gland) (HeW) and gonad weight (GoW) were recorded. Wet weight (0.1 mg) of stomach content (ScW) was also recorded after plastic screening.

In order to prevent contamination, all material used (scissors, tweezers and Petri dishes) was rinsed multiple times with filtered water (50 μm metal sieve) and checked for contamination before use. Cotton lab coat and nitrile gloves were worn at all times. Moreover, an isolation device adapted from the one proposed by Torre et al. (2016) was used to cover the stereomicroscope and work area throughout the screening and characterization process. Procedural controls, that is open Petri dishes filled with filtered water, were placed inside and outside of the isolation device during digestive content screening in order to assess potential airborne contamination. Only fiber shaped items were found in both controls. Contamination found in the inside controls (average values of 0.25 fibers per digestive content screened) was four times less abundant than contamination in outside controls, thus pointing out the efficiency of our isolation device in reducing potential contamination. Fibers found in inside controls were visually similar to cellulosic fibers, yet they were short (<0.5 mm), clean and always appeared on the surface of the water (pointing out that they were deposited from air). Therefore, fibers from digestive contents were only counted if they were clearly embedded in the digestive content and/or with detritus attached and never when floating on the surface. Because of that, no correction factor was applied to the final values of fibers reported.

Environmental and anthropic variables related to plastic sources or pathways into the deep sea were collected afterwards including: mean river flow (mean value of the water flow at the closest gauging station to the river mouth possible, in $m^3\ s^{-1}$), total accumulated precipitation (precipitation accumulated over the last 3 months at the nearest meteorological station, in mm), fishing pressure (sum of power in kW of the fishing fleet estimated to be operating in the area), population density (mean values of habitants km^{-2} of the coastal area region) and land cover (urban area, agricultural land and forests in percentage per region). Data was provided by Agència Catalana de l'Aigua (ACA, 2019), Confederación Hidrográfica del Ebro (SAIH, 2019), Servei Meteorològic de Catalunya (Meteocat, 2019), Departament d'Agricultura, Ramaderia, Pesca i Alimentació (DARPA, 2019), and Institut d'Estadística de Catalunya (IDESCAT, 2019). The shortest distance to the coastline (in km) was calculated for each sampling point using QGIS 3.0.3.

2.2. Characterization of anthropogenic particles

Anthropogenic particles were separated, counted and classified into isolated fibers or ball-forming fibers. Only in one occasion an anthropogenic particle other than a fiber or a ball of tangled fibers, a film-like particle, was observed. Its colour and size (area) were recorded but it was not included in further analysis. Isolated fibers were cleaned (organic material attached was removed carefully with needles), mounted in distilled water and observed by light microscopy. Total length and mean diameter (calculated on three random measures along the fiber) were measured, and fibers were then classified into micro- (<1 mm), meso- (1–5 mm) or macro-sized fibers (>5 mm) and their colour was recorded. Moreover, fibers were classified into five categories according to visual aspects such as diameter uniformity and cross-section, finishing, striations and signs of wear and surface and backbone texture, following a similar approach to that used in forensic sciences (Bell, 2006) (Supplementary Material, Table S1).

When balls occurred (aggregation of entangled fibers) images were taken without untangling them or entirely removing organic debris (only that loosely attached) in order to estimate the occupying area (BA in mm^2). Afterwards, balls were carefully unraveled as much as possible and all newly separated fibers were measured and characterized as stated above for originally isolated fibers (except for the fact that diameter was only measured in 20 randomly selected fibers per ball, and that they were not classified into micro-, meso- or macro-sized fibers). When complete isolation was not possible, the remaining tangles were mounted and the number of fibers, its composition (in proportions) and total length were estimated. Balls were categorized into four categories according to their morphology and size (in terms of the number of fibers and sum of the length of all constituent fibers (TL)) (Table 2). Finally, ball density (BD) was estimated for each ball as BD = BA / TL in mm.

Images and measures were taken using a ProgRes® C3 (JENOPTIK Optical Systems GmbH, Germany) coupled to a Leica DM500B microscope. Up to seventeen colours were reported, but given the low prevalence of some of them, they were grouped into six categories (transparent, blue, red, other bright, other dark, and black).

Fourier-Transformed Infrared Spectrometry (FTIR) was carried out on a randomly selected subsample of 119 anthropogenic fibers (2.9% of the total of fibers screened). This random selection was weighted by the relative abundance of fibers in each sampling, thus including at least a 4% of the fibers found at each sampling point (except for one, where the abundance was over ten times higher than in the rest). Spectra were recorded using a Tensor 27 FTIR spectrometer (Bruker Optik GmbH, Germany) equipped with a diamond attenuated total reflectance (ATR) unit (16 scans cm^{-1}, 800-3600 cm^{-1}). Resulting spectra were treated (baseline corrections, peak normalization and selection of characteristic band applied) with Spectragryph 1.2.11 (Menges, 2019) and compared with reference spectra (custom-made library of common polymers, Carreras-Colom et al., 2018). Successful identification was considered for similarity values above 70%. The percentage of polymers identified for each category of fibers was calculated and used to determine the polymer composition of the rest of the visually sorted fibers (Supplementary Material, Table S1). Distinction between artificial (e.g. rayon) and natural (e.g. cotton) cellulosic fibers was not possible. Fibers were classified into anthropogenic fibers (including both cellulosic and synthetic fibers) and synthetic fibers (excluding cellulosic fibers).

2.3. Shrimp's health condition

A portion of hepatopancreas and gonad, as well as a portion of

Table 2
Visual classification of balls (aggregation of tangled up fibers) based on three main criteria: morphology, number of constituent fibers and sum of the length of all constituent fibers (TL).

Type	Description	Constituent fibers	TL (mm)
B-I	Very loose ball. Sometimes aggregation depends strongly on attached organic matter. No core. Easy to untangle.	<5	<50
B-II	Medium-loose ball. Loose core. Can be easily unraveled (sometimes only braided).	10–20	50–100
B-III	Tight ball. Not only braided but also with several knots. One tight core. Difficult to unravel.	30–50	100–200
B-IV	Tight large ball. More than one tight core or an extended one, with several complex knots. Impossible to untangle completely without breaking fibers.	>50	>200

muscle of each individual (n = 201) and the stomach and intestine walls from selected individuals (n = 21) with the greatest or the lowest values of plastic pollution, were processed through routine histologic techniques. Qualitative histological examination by light microscopy was conducted for different organs (muscle, gills, hepatopancreas, gonad and digestive tract – stomach and intestine). Normal structures reported in other shrimp species were used as a basis (Bell and Lightner, 1988). Sexual maturity was also determined according to Carbonell et al. (2006).

Shrimp's health condition was further assessed by relative condition index (as Kn TeW/EW× 100, EW being the expected weight estimated from a length-weight relationship considering all data) (Le Cren, 1951), hepatosomatic index (HSI =HeW/TeW × 100) and gonadosomatic index (GSI = GoW/TeW × 100). Feeding intensity was measured through a fullness stomach index calculated as follows F =ScW/TeW × 100. A second visually determined fullness index was also assigned to each individual from 0% (empty stomachs) to 100% (full stomachs) according to the volume of the content.

2.4. Data analysis

Fiber load was calculated for each individual and organ (stomach/intestine) in terms of total abundance (TA, as the sum of all estimated fibers) and total length (TL, as the sum of the length of all fibers) including all anthropogenic fibers. A corrected fiber load in terms of total length including only synthetic (i.e. plastic) fibers (TLs) was also calculated for each individual. Occurrence of fibers (FO) and balls (BO) was calculated for each sampling point as the number of individuals with anthropogenic fibers or balls/total number of individuals x 100.

Spatial, seasonal and temporal differences in the occurrence of fibers (FO) and balls (BO) were explored by means of Pearson's Chi-square tests followed by Fisher's exact test for pairwise tests. Differences in fiber load (in terms of TA, TL, TLs) and ball size (in terms of BA and BD) were analyzed using Kruskal-Wallis tests coupled with Dunn's post-hoc tests (as data failed to meet normal variance structure). Similarly, spatial, seasonal and temporal (over a 10 year period) differences in the composition of fibers, in terms of organ location, size category (for isolated fibers only), colour and polymer, and ball type were explored through PERMANOVA analysis performed in PRIMER PERMANOVA+6 (Anderson et al., 2008). Permutation p-values were obtained under restricted permutation of raw data (9999 permutations) performed on Bray-Curtis similarity matrices derived from square-root transformed data. In all cases, proportions for each category were used instead of absolute values of abundance or length. A similarity percentages analysis (SIMPER) was carried out afterwards to identify the fiber-related variable that contributed the most to the similarity/dissimilarity of samples.

Correlation amongst detailed descriptors of fiber load (fibers in each location – stomach or intestine content – and according to their size – micro-, meso- and macro-fibers –) with other biological and environmental variables were explored through Cramer's coefficient and Spearman's correlation. Generalized linear models (GLM) were used to evaluate how environmental, anthropic and biological variables were related to the occurrence of balls (BO; considered more informative than fiber occurrence) and the total abundance of fibers (TA) in shrimps. BO (as a binary presence/absence variable) and TA individual data was related to location, year, season, CL, K, HSI, F, depth, distance to coastline, accumulated precipitation, river discharge, population density, fishing pressure and land cover. Following Burnham and Anderson (2002), the most parsimonious models were selected using the lowest AIC (Akaike's Information Criterion). Similarly, effect of the fiber load on health condition of shrimps was explored through GLM in which condition indices (Kn, HSI, GSI – only for females –) were the response variables and fiber load (TA, TL, BA and BD) the explanatory variables. Shrimp's size, stomach fullness and repletion index, sexual maturity and environmental variables (year, season and locality) were also included as explanatory variables to account for other sources of variability. The best model was used to explore negative effects of fiber load on condition indices. Models were fitted for each sampling point separately when interactions between year, season or locality were found.

Statistical analysis was carried out using R software, version 3.5.3 (R Foundation for Statistical Computing) (packages vegan, dunn.test, lme and MASS) if not indicated otherwise. Significance levels were fixed at 0.05 for each statistical hypothesis testing.

3. Results

All individuals examined were adults, mostly sexually mature females (stages III-IV in spring; stage IV-V in summer), with sizes (CL) ranging between 22.46 and 47.43 mm. From the total of 201 individuals of *Aristeus antennatus* screened, 151 (75.12%) were observed to contain at least one plastic fiber and 44 (21.9%) had tangled balls of fibers in the stomach. Only once was a ball (5 fibers) found in the intestine Overall, more than 65% of shrimps from each sampling point had fibers of some sort (isolated or tangled, in stomach or intestine contents) (Fig. 1 and Supplementary Material, Table S2 and Table S3) and only one shrimp had a film-like blue particle (*ca.* 1.2 mm^2) in the stomach. Fibers found exhibited a great range of lengths (from 0.16 to 37.9 mm), widths (from 0.006 to 0.168 mm), colours (up to seventeen) and polymer types, including polyester (49.7%), acrylic (27.5%), polyamide (12.4%), polypropylene (8.6%) and cellulosic (1.8%) fibers. It should be noted that cellulosic fibers counted in digestive contents differed from those of inside controls (airborne contamination) in that they were longer (average values of 3 mm per fiber compared to fibers of <0.5 mm in controls), were observed embedded in digestive content and/or with detritus attached to their surface, and were sometimes unraveled from tangled balls of fibers (clearly not airborne contamination).

Fibers were more common and abundant in stomach contents (FO = 78.1%; TL = 114.23 mm/individual) than in intestine contents (FO = 44.3%; 4.62 mm/individual). Even though there was a positive significant correlation between fiber load in stomach and intestine contents (ρ = 0.283 and ρ = 0.230 for TA and TL respectively), fibers found in the stomach accounted for >94% of all fibers identified. Almost all isolated fibers encountered measured >1 mm (96.7%) with meso-sized fibers (1–5 mm) being the most abundant size class in both stomach and intestine contents (55.4% and 73.8% respectively). Macro-sized fibers (>5 mm) were the second most abundant size class representing 42.0% of the isolated fibers in stomachs and 21.0% in intestines.

3.1. Differences in fiber ingestion between locations, years and seasons

With regards samples collected in 2018, both spatial and seasonal differences were found (inset. Fig. 1, Supplementary Material, Table S2). During spring there was a trend towards a greater load of fibers to the south, especially in the form of stranded fibers, with the highest values of ball occurrence, area and density in the Ebro Delta area (BO: X^2 = 16.939, p = 0.0002; BA: X^2 = 14.582, p = 0.0007; BD: X^2 = 16.78, p = 0.0002). On the other hand, fiber load in summer was higher in shrimps off Barcelona compared to the other localities in terms of TA (X^2 = 27.274, p < 0.001), TL (X^2 = 29.095, p < 0.001) and TLs (X^2 = 29.567, p < 0.001) (inset Fig. 1.). Ball occurrence and size was also higher off Barcelona (BO,

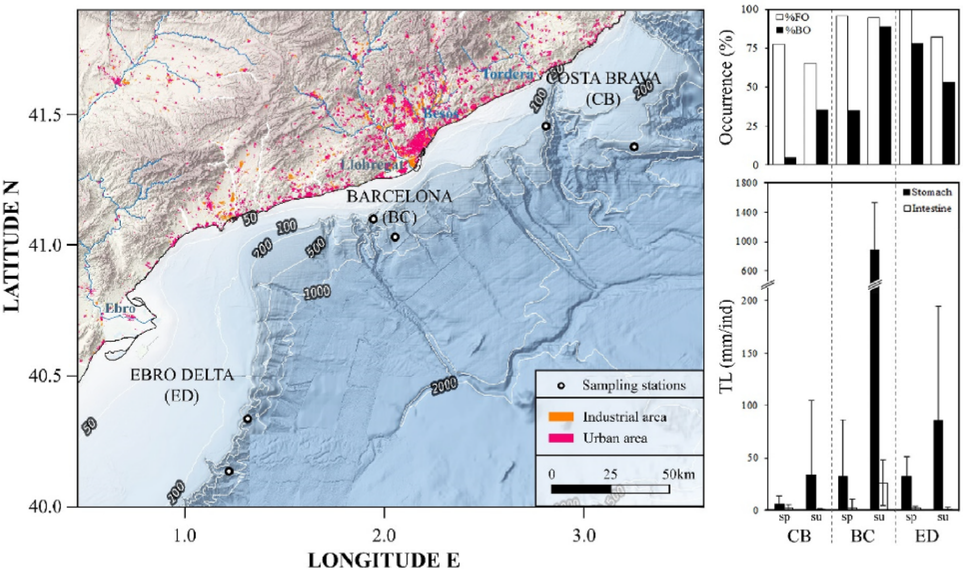


Fig. 1. Map of the study area showing the occurrence of fibers and balls and the mean total length of fibers (TL) per individual in *Aristeus antennatus* captured along the Catalan coast in spring (sp) and summer (su) of 2018. Occurrence values in percentage of individuals are displayed in the top right corner with vertical bars in white fill colour for all fibers (isolated or tangled) and black fill colour for balls (tangled fibers). Mean values (± standard error) of TL in mm per individual are depicted with vertical bars in white fill colour for fibers in the stomach and dark fill colour for fibers recovered from the intestine content. Main rivers (Tordera, Besòs, Llobregat and Ebro) and industrial (orange) and urban (magenta) areas are also represented. The background map is from EMODnet Bathymetry Consortium (2016): EMODnet Digital Bathymetry (DTM). https://doi.org/10.12770/c7b53704-999d-4721-b1a3-04ec60c87238. Land and urban areas data reprinted from SIOSE [www.siose.es] under a CC BY licence, original copyright 2016. River data reprinted from [aca.gencat.cat] under a CC BY licence, original copyright 2012. (For interpretation of the references to colour the reader is referred to the web version of this article.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$X^2 = 11.585$, $p = 0.003$; BA, $X^2 = 28.358$, $p < 0.001$; BD, $X^2 = 14.707$, $p = 0.0006$), and there was a greater occurrence of complex balls (B-IV, $X^2 = 18.462$, $p = 0.0052$; Fig. 2.). Shrimps from this sampling station (off Barcelona city just south of the Llobregat River, P2V) showed the highest fiber loads throughout the study with mean values of fiber load per individual ten times higher than shrimps from other locations or periods (inset Fig. 1, Supplementary Material, Table S2). A total of 16.5 lineal meters of fibers (mean diameter 0.025 mm) were recovered from 18 individuals and the maximum load of fibers found in a single shrimp was 2.35 m (of which at least 98% was plastic).

Shrimps from Barcelona in 2018 also showed the greatest seasonal (spring vs summer) shift in fiber load (over twenty times higher values of TA and TL, and balls twice as frequent and dense), whereas in Costa Brava and the Ebro Delta areas, only minor differences between seasons were found: a significant decrease in TA and TL of fibers in intestine contents (Costa Brava: TA, $X^2 = 10.436$, $p = 0.0012$; TL, $X^2 = 9.0985$, $p = 0.0025$; Ebro Delta: TA, $X^2 = 6.7036$, $p = 0.0096$; TL, $X^2 = 3.5061$, $p = 0.061$; inset Fig. 1., Supplementary Material, Table S2) and an increase in BO (only in Costa Brava, Fisher's exact test, $p = 0.018$). It should be noted that only one shrimp from spring samplings in Costa Brava had a ball of fibers in the stomach, the lowest percentage of BO throughout the study.

In terms of fiber characterization, differences in polymer, colour and size composition were found between locations in 2018. In spring, shrimps from Costa Brava showed a different polymer composition compared to the rest of localities ($p = 0.0125$, 9947 unique perms) with a higher proportion of cellulosic fibers. In summer, shrimps from Barcelona presented a more marked dominance of PET and acrylic fibers (Fig. 3). With regards colour, differences were only found between the Costa Brava and Barcelona areas (spring: $p = 0.0021$, 9948 unique perm.; summer: $p = 0.0024$, 9948 unique perm.) with a larger proportion of coloured fibers in Barcelona. The mean proportion of each colour category was between 4 and 8%, except for transparent and translucent fibers accounting for nearly 60%, whereas in Costa Brava transparent alongside black (spring) and blue (summer) were the dominant colours (Fig. 4). Finally, differences in size composition were only found between Barcelona and the Ebro Delta areas in spring ($p = 0.046$, 9959 unique perms) and between seasons for both shrimps from the Ebro Delta ($p = 0.0474$, 2645 unique perms) and the Costa Brava ($p = 0.0096$, 979 unique perms) with a significant increase in the proportion of >5 mm fibers in summer.

Analyses on shrimps from different years (2007, 2017 and 2018) showed a great difference between summer samples from 2018 and the others, with values of fiber load (TA and TL) in 2018 nearly thirty times the values from summer 2007 (Fig. 5, Supplementary Material, Table S3; TA: $X^2 = 22.003$, $p < 0.001$; TL, $X^2 = 21.928$, $p < 0.001$). On the other hand, shrimps sampled in 2017 (spring) showed lower values of fiber load compared to either 2007 (TL $X^2 = 6.6151$, $p = 0.01$) and 2018 (TL $X^2 = 4.7172$, $p = 0.03$) samplings (Fig. 5, Supplementary Material, Table S3), yet values were in the same order of magnitude. The seasonal trend observed in 2018 (ten-fold increase in summer compared to spring) was not observed in 2007. No significant differences in isolated fiber's size or fiber colour composition were found between past (2007) and present (2017, 2018) years, either. Only a significant change in the

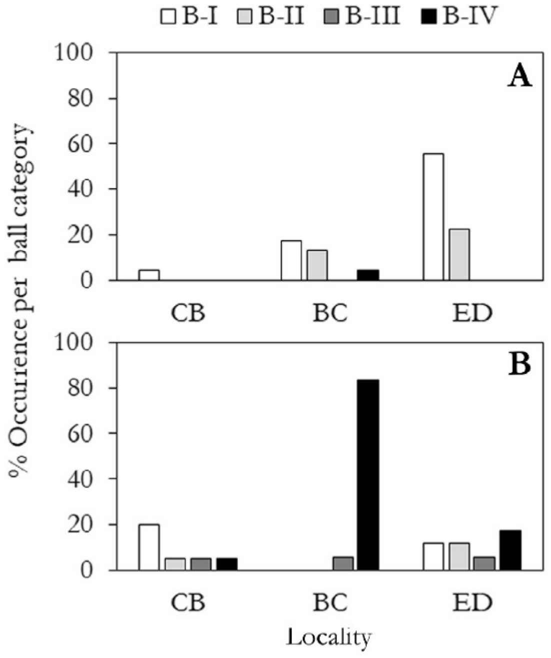


Fig. 2. Spatial (three localities: CB – Costa Brava, BC – Barcelona, ED – Ebro Delta) and seasonal (A – spring, B – summer) values of ball occurrence according to their category (form small and simple to big and complex: B–I, B-II, B-III, B-IV).

polymer composition was observed (spring: $p = 0.0287$, 9950 unique perms; summer: $p = 0.011$, 9937 unique perms), with a

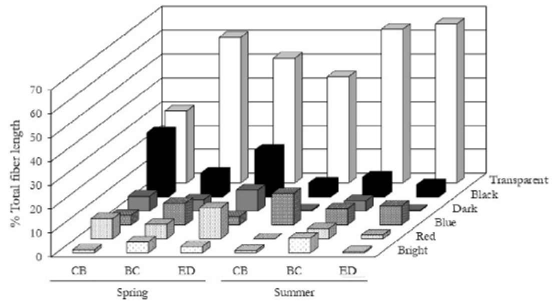


Fig. 4. Colour composition of fibers recovered from individuals of *Aristeus antennatus* along the Catalan coast (off Costa Brava - CB, off Barcelona city – BC, and off the Ebro's Delta - ED) during spring and summer of 2018. Dark category included from green to purple dark colours others than black and blue. Bright category included yellow to turquoise bright colours others than red or bright blue.

significant shift in the proportion of acrylic and PET fibers (Fig. 6).

3.2. *Factors related to fiber load in Aristeus antennatus*

All total fiber load descriptors (TA, TL, BA and BD) were highly correlated ($\rho > 0.8$). Spearman's correlation coefficient decreased when the location (intestine or stomach; $\rho < 0.3$) and size (<1, 1–5 and >5 mm) of fibers encountered was considered ($\rho < 0.33$). Size of balls (in terms of BA, BD and TL of tangled up fibers) was positively correlated with the abundance of meso- (1–5 mm; $\rho = 0.356$) and macro-sized (>5 mm; $\rho = 0.269$) isolated fibers in the stomach. The abundance of stomach fibers (sizes 1–5 and >5 mm) was weakly ($\rho = 0.210$ and $\rho = 0.187$, respectively) but significantly correlated to stomach fullness. No other significant correlations with shrimp size, fullness or condition indices were found.

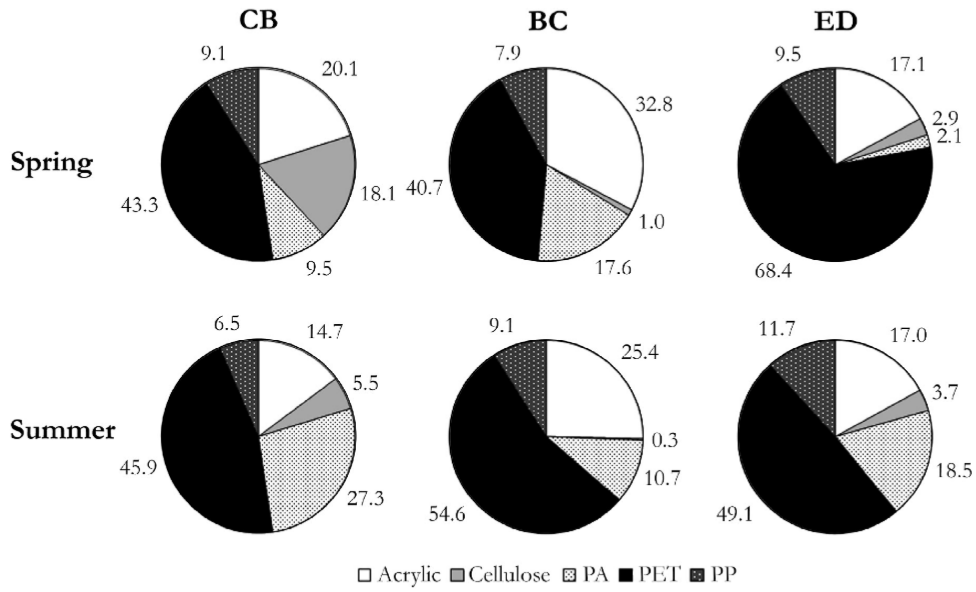


Fig. 3. Polymer composition of fibers recovered from *Aristeus antennatus* sampled along the Catalan coast (off Costa Brava (CB), off Barcelona city (BC) and off the Ebro Delta (ED)) in spring (top line) and summer (bottom line) in 2018. PA – polyamide; PET – Polyethylene terephthalate; PP – polypropylene.

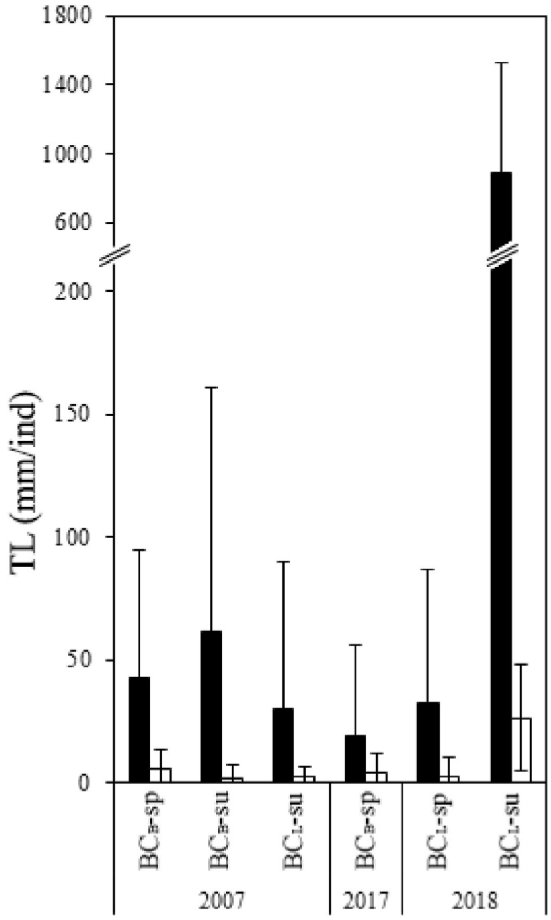


Fig. 5. Mean values (±standard error) of fiber load (TL) per individual of *Aristeus atennatus* captured in the area off Barcelona (BC) in spring (sp) and summer (su) of 2007, 2017 or 2018. See Table 1 for details on the sampling.

The results of the GLM identified that both the likelihood of BO and the values of TA in *A. antennatus* were mostly related to environmental and anthropic variables rather than biological ones. The best-fitting model for BO (AIC = 231.46) included trawl depth, population density, season and location. Individuals were more likely to contain tangled balls of fibers in areas with higher population density ($z = 3.432$, $p < 0.001$) and at greater depths ($z = -4.440$, $p < 0.001$). Also, shrimps collected in summer and from the Delta area were more likely to contain balls ($z = -3.124$, $p = 0.002$, and, $z = -3.524$, $p < 0.001$, respectively); however, the influence of these covariates on BO was smaller than trawl depth and population density. The second best-fitting model (AIC = 233.44) also included accumulated precipitation as a co-variate yet its influence on BO was not significant ($z = -0.140$, $p = 0.88$). Analysis of factors related to TA showed a similar outcome, with the best-fitting model again including location, depth and population density ($p < 0.001$) in addition to year, fishing intensity and CL (the only biological variable included in any model). Higher values of TA were more likely to occur in shrimps collected in 2007, yet the magnitude of influence of this covariate was the smallest in this model. Shrimps with a smaller CL ($p < 0.001$) had low values of TA; which could be driven by the fact that shrimps from Blanes, where the lowest values of fiber load were found, also were some of the smallest individuals analyzed. Similarly, the negative relationship observed between TA and fishing intensity ($p < 0.001$) could be driven by the fact that extremely high values of TA were found in Barcelona, where fishing intensity is lower.

3.3. *Relationship between fiber ingestion and shrimp's health condition*

Model selection showed that most of the models exploring the relationship between condition indices (Kn, HSI and GSI as response variables) and fiber and ball occurrence and fiber load were best fitted without fiber-related variables, with the variability in condition indices being mostly related to CL and season. HSI was higher in spring samplings (average values ranging 2.96–5.72 in spring compared to 6.29–8.02 in summer, $p < 0.05$), whereas GSI values were higher in summer (0.38–1.63 compared to 3.11–6.67, $p < 0.05$), except for shrimps from the Ebro Delta (Supplementary Material, Table S4). These differences were more pronounced in bigger, older and more mature individuals. No differences were observed for Kn in relation to CL nor season. A significant negative relationship between fiber load (TL) and GSI was found ($F_{1,16} = 10.77$, $p = 0.0047$, $R^2 = 0.3649$) only for P2V sampling (off Barcelona, summer 2018). Shrimps with the highest load of fibers (150 cm individual^{-1}) showed values of GSI about 60% lower compared to those shrimps with the smallest load of fibers (5 cm individual^{-1}).

No histological changes or alterations from the normal tissular pattern in this species were observed in the organs and tissues examined, not even in those supposed to be in direct contact with balls of tangled fibers such as the cuticle and the epithelium of the digestive system. In addition, no parasites or relevant histopathological findings were found in this study.

4. Discussion

Fiber ingestion by the deep-sea shrimp *Aristeus antennatus* is reported from every sampled location and period reinforcing the idea that it is a widespread and recurrent phenomenon that dates back more than ten years for this species in the Catalan Sea (NW Mediterranean Sea). Extremely concerning values for shrimps caught off Barcelona have been observed, with values of over 1 m of accumulated fiber length in at least seven individuals. As already pointed out in a previous work in this species (Carreras-Colom et al., 2018), fiber ingestion might be the result of intended or unintended consumption while feeding on benthos where fibers might be present following sedimentation.

Our results show high values of fiber occurrence 84.6% (78.1% when only considering the stomach) and ball occurrence (42.4%) very similar to those reported by Murray and Cowie (2011) for *Nephrops norvegicus* in the Clyde Sea (83% of fiber occurrence and 50% of ball prevalence in stomachs). The values for both stomach fiber and ball occurrence in shrimps captured off Barcelona in summer of 2007 and 2018 were higher than those found in the same area in summer of 2010 (Carreras-Colom et al., 2018; 25.9% of fiber occurrence and 3.7% of ball occurrence). These higher values could be due to either differences in depth (512–791 m this study and about 1000m in the previous one) or to great variability in the environmental concentrations of anthropogenic fibers, and thus availability to shrimps.

Presence of fibers in the intestine is reported for the first time in

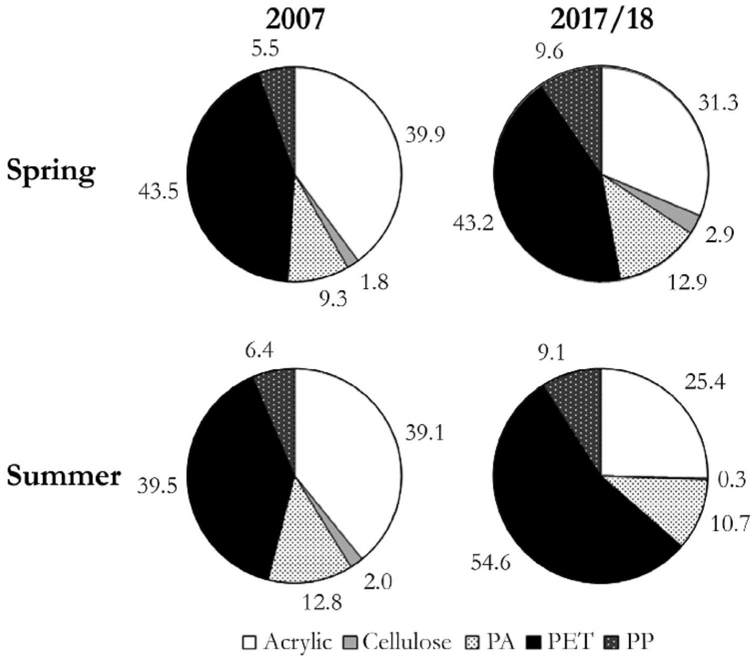


Fig. 6. Polymer composition of fibers recovered from individuals of *Aristeus antennatus* off Barcelona in different periods (2007 - left, 2017/18 - right) and seasons (spring – top; summer – bottom). Values from different localities (in front of the Besòs river mouth and just south from Llobregat's river, see Table 1 for details) were grouped and the mean value is depicted. PA – polyamide; PET – polyethylene terephthalate; PP – polypropylene.

A. antennatus. According to our results, fiber loads are much lower in the intestine than in the stomach suggesting that this route of egestion might be relatively unimportant. Given the length of most of the isolated fibers (97.4% > 1 mm and 42.0% > 5 mm), not to mention the size of tangled balls of fibers, passage through the pyloric stomach into the intestine is thought to be limited due to the narrowing at the entrance of the intestine and the action of the gastric mill (Welden and Cowie, 2016b). Saborowski et al. (2019) observed that microbeads and small fibers (<100 μm) were passed through the stomach and into the gut in individuals of *Palaemon varidans*, whereas long fibers (>100 μm) could not be transferred and remained in the stomach. Moreover, longer fibers might be more prone to be knotted into balls by the action of the gastric mill's, increasing the overall size and thus hampering passage down the gut (Welden and Cowie, 2016b). On the other hand, the action of the gastric mill combined with the cutting effect of numerous shell remains (from prey like bivalves or pteropods, common items found as part of *A. antennatus* diet (Cartes, 1994),) could also facilitate, to a lesser degree, plastic egestion by breaking down fibers (Watts et al., 2015). Ultimately, moulting is the most plausible route of significant fiber elimination. During ecdysis, the stomach of decapods, including the gastric mill, is replaced and expelled. Welden and Cowie (2016b) confirmed the presence of microplastics inside the stomach linings of *N. norvegicus* previously fed with microplastics and the absence of microplastics in the stomach of moulted individuals.

The nearly exclusive presence of fibers in *A. antennatus* is in accordance with results reported for other crustaceans such as the brown shrimp, *Crangon crangon*, from the North Sea (Devriese et al., 2015) and the Norway lobster, *N. norvegicus*, from the Clyde Sea (Murray and Cowie, 2011; Welden and Cowie, 2016b). In all these studies, most of the items found were fibers with only some pieces of films and a granule being identified. Besides being more easily ingested and retained, the predominance of fibers in the environment could also explain this bias. In a recent review, Gago et al. (2018) suggested that fibers are more abundant than other shapes in seawater and sediments. Though this statement should be treated with caution as values obtained through very different methodologies were compared, fiber predominance is supported in our study area as separately reported for sediments (Sanchez-Vidal et al., 2018) and several pelagic and demersal fish species (Bellas et al., 2016; Compa et al., 2018; Nadal et al., 2016).

Hydrodynamic processes such as dense-shelf water cascading, severe coastal storms, offshore convection and saline subduction, known for their importance on the transport of sediments and organic matter to the deep sea (Canals et al., 2006), are also key processes on the transport of fibers to deep areas where they accumulate and become available for deep-sea organisms like *A. antennatus* (Bagaev et al., 2017; Woodall et al., 2014). These above-mentioned hydrodynamic processes have been pointed out as the main factors explaining marine litter occurrence along spatial gradients in the NW Mediterranean Sea (Tubau et al., 2015). In particular, the Northern Current, which flows southwards along the edge of the continental shelf (Font et al., 1988), might help explain the trend towards an increased fiber presence in shrimps towards the south during spring. The potential of the Northern Current in leading to a progressive accumulation of microplastics along its path was already pointed out by de Haan et al. (2019), whose study reported higher values of microplastic particles in Catalan surface waters (0.183 ± 0.158 items m^{-2}) compared to those reported in the Gulf of Lions (0.08 ± 0.03 items m^{-2}; Pedrotti et al., 2016). In addition, local sources of fibers could also be more abundant, especially in the area off Barcelona, near to densely populated areas, but also off the Ebro Delta, which might receive an indirect influence of human activities through the discharge of the Ebro River. Galimany et al. (2019) attributed higher densities of plastic litter in shallow (5.6–67.7 m) fishing grounds near Barcelona (~60 kg km^{-2}) compared to the Ebro Delta (<5 kg km^{-2}) to inland mismanagement and riverine outflows being more intense in the former. Similarly, values of marine litter reported by Galgani et al. (1995) and García-Rivera et al. (2018) were also higher near Barcelona compared to other areas of the Catalan coast. However, studies reporting environmental concentrations of microplastics in the area are either scarce or hardly comparable (Supplementary Material, Table S5), especially regarding the deep-sea environment. So far, only one study has been conducted in the Blanes area, targeting sediments of the submarine canyon and its adjacent slope, with values of microplastic fibers ranging between 5,000 and 12,000 fibers m^{-2} (about 70% of them being cellulosic fibers). For the sampled areas off Barcelona and the Ebro Delta only values of either microplastics in surface waters or coastal and beach sediment can be found in the literature, which are hardly relatable to plastic ingestion in shrimps.

The great difference in fiber load (ten-fold increase) observed between spring and summer samplings from Barcelona is believed to be caused by the increase in environmental concentrations leading to increased ingestion by shrimps. This increase should have occurred in a rather short period of time which, in addition to a significant increase mostly of long fibers (>1 mm), would suggest that pollution sources were close (Isobe et al., 2019). Moreover, in the NW Mediterranean, flux of (total) particles arriving at bathyal depths (to 1000 m) near the bottom are important in spring and peak at the end of June, just before the peak observed for fibers in shrimps in July, decreasing later in summer (Miquel et al., 1994). de Haan et al. (2019) also observed a great variability along the Catalan coast finding both their lowest and highest microplastic abundances (0.01 items m^{-2} and 0.5 items m^{-2}) only ~60 km apart and during the same period of the year.

Fiber sources include clothing (Browne et al., 2011), polymer manufacturing and processing industries (Lechner and Ramler, 2015). Most of the research concerning microplastics has been focused on the first one, with several studies pointing at the shedding of fibers while washing clothes as a major source of microplastics into the ocean (Salvador Cesa et al., 2017). However, recent works focused on the efficiency of wastewater treatment plants have shown that they actually have an efficient performance on removing fibers thanks to the tendency of fibers to mix intimately with the cellulosic matrix of the influents and aggregate into flocs that can be easily retained in sieves (Carr et al., 2016). Therefore, even though fibers might escape wastewater treatment plants to some extent, this source might not contribute as much as it was thought initially (Carr, 2017; Carr et al., 2016). Alternative significant dispersive pathways would include atmospheric fallout, household dust, wastewater treatment plants disposals and stormwater runoff (Dris et al., 2017; Siegfried et al., 2017; Wagner and Lambert, 2018). The latter could play a key role in the Mediterranean area where the regime and hydrography are characterized by seasonal storm-like events leading to flash floods (Tubau et al., 2015). These abrupt increases in rainfall can also occasionally lead to combined sewer and storm-water overflows with a large impact on the receiving coastal waters. Increased river discharge usually creates great plumes in river mouths revealing the great amounts of sediments transported in these events. There is an absence of studies in the area on the specific occurrence of microplastics during these events, yet given that all Catalan rivers drain well-urbanized watersheds presence of fibers could be expected (de Haan et al., 2019; Sanchez-Vidal et al., 2013; Tubau et al., 2015). The precipitation regime in 2018 was extraordinary, especially in the Barcelona area (984.2 mm by the end of the year compared to the mean value of 580.6 mm for the past 10 years (Meteocat, 2019)), which might explain the great values of fiber load found in shrimps from that location in summer (considered an outlier as they were 10–30 times higher than fiber loads observed in other locations). An increase in the C/N ratio (~10), indicative of continental inputs in the sedimented organic matter, was also reported in the same area of study (off Barcelona at the head of the Besòs Canyon at 600 m) after 2–3 months following the maximum river discharge (Rumolo et al., 2015). Similar fluctuations on the occurrence of plastics after increased rainfall episodes have been reported in estuary environments (Dantas et al., 2012; Lima et al., 2014) and the Turkish Mersin Bay where a 14-fold increase was observed (Gündoğdu et al., 2018). In the latter, a significant change in the polymer composition was also observed, with up to eight new different polymers being identified in the post-flood period. In our results, only a slight increase in the proportion of PET and acrylic fibers, which were already the most abundant categories, was detected.

The great increase in fiber load observed in summer (compared to spring) in shrimps from Barcelona in 2018 was not identified in any of the other seasonal comparisons, neither in the same area (2007) nor in other localities (2018). Possible explanations regarding 2018 samples for the northernmost area (Costa Brava) might be the lower input throughout the year, as a site with less anthropogenic pressure, while in the southernmost area (Ebro Delta) it could be related to the time of sampling (in late summer, nearly 1.5 months after the summer samplings in other localities). This short period of time might have been enough for a significant proportion of the shrimp population to moult and get rid of the highest concentrations acquired in late spring and early summer. Unlike other crustaceans that brood eggs and for which moulting is limited to certain periods, *A. antennatus* shows high proportions of individuals moulting throughout the year and especially during summer (values over 50% of the population) in which moulting optimizes fertilization (Demestre, 1995). Since moulting activity is so intense in summer and considering that moulting implies a complete or at least significant loss of fibers in the stomach, the idea that shrimps from Barcelona acquired fibers rapidly owing to an exposure to high concentrations in the environment is reinforced.

Our results on plastic ingestion in shrimps from different periods (2007 compared to 2017 and 2018), with the exception of one sampling considered an outlier (Barcelona, summer 2018), suggest that the level of fiber ingestion in that particular location (off Barcelona) could have remained at a similar level (between 22.5 and 62.37 mm ind^{-1} in average). Beer et al. (2018) also reported no changes in the average values of plastic ingestion in planktivorous fishes from the Baltic Sea across three decades. These results seem unexpected given the increase in global plastic and fiber production (Geyer et al., 2017; Jambeck et al., 2015) and point out the need to better understand the fate of anthropogenic fibers once they enter marine ecosystems. In individuals sampled monthly from Barcelona and Costa Brava for diet studies back in 1988–1989, the occurrence of fibers and balls was also noted (Cartes, 1994), yet not described in detail. Although the study was mostly focused on identifying the items – prey – found in stomach contents and measures to prevent airborne contamination for fibers were not adopted (so, fiber occurrence could be overestimated) occurrence of fibers in shrimps from off Costa Brava (32.1%) was slightly lower than in Barcelona (42.9%), as reported in our study. Moreover, balls, which are unlikely to come from airborne contamination, showed the same spatial pattern described in this study for 2018 spatial trends, with a clear higher occurrence off Barcelona (19.8%) than off

Costa Brava (7.1%) (Supplementary Material, Fig. S1.). Similarly, Carreras-Colom et al. (2018) reported the highest values of fiber and ball occurrence near Barcelona (52.1% of fiber occurrence and 18.3% of ball occurrence for the period 2008–2011) in a survey in the Catalan Sea. These findings, together with the relevance of population density as a covariate in our models predicting the likelihood of balls and the fiber load, support the idea that the area off Barcelona is, and has probably been for the past years, an impacted area for microplastics. More studies, focusing on environmental concentrations or plastic ingestion in other species, are needed to draw more definite conclusions on temporal trends for specific fiber loads, yet it seems clear that at least the occurrence of fiber ingestion in this species has remained rather high (>40%) throughout the past ten years. Furthermore, we did find a significant shift in the polymer composition, which might be related to a shift in production and usage trends. Shrimps caught in 2007 showed a greater proportion of acrylic fibers whereas in 2017–2018 the most common type was polyester. The global acrylic fiber market has declined in recent years, especially in Europe, in favour of polyesters, which have a price advantage thanks to large-scale production, better raw material availability, and recyclability (HIS Markit, 2016).

In general terms, the synthetic polymers identified and their contribution (in order of predominance: polyester, acrylic, polyamide and polypropylene), except for the lack of polyethylene, are in accordance with those reported in other studies encountering fibers in the environment (Browne et al., 2011; Murphy et al., 2016; Sanchez-Vidal et al., 2018). The highest proportion of cellulosic fibers in shrimps was found in the Costa Brava area, where Sanchez-Vidal et al. (2018) particularly reported the dominance of cellulosic fibers over other synthetic polymers. Cellulosic fibers, which can be natural (e.g. cotton) or artificial (e.g. rayon), were found in a low proportion overall and were not eliminated from analyses as they were sometimes observed to be part of balls thus posing a physical threat to food passage and ultimately shrimp's health. In fact, balls were composed of a diverse suite of fibers from different colours, sizes (length and diameter) and even polymers, rather than being consistent in appearance (Rochman et al., 2019). This diverse composition, together with their morphology, with some balls seemingly been made up of other small bundles, might suggest that their origin is diverse.

The general condition of shrimps, as assessed through condition indices and histology of main organs, showed no consistent negative impact of fiber ingestion nor any sign of other potential stressors (i.e. prolonged starvation, extreme environmental conditions). Histological alterations such as inflammatory responses or alterations of the epithelia as the ones reported in experimental exposures to polyethylene microplastics (Rodriguez-Seijo et al., 2017; Von Moos et al., 2012) were not observed. Variability on body indices, especially HSI and GSI, was mostly related to season and shrimp's size which are, indeed, related to the ecology of the species (feeding rate and reproduction according) (Cartes et al., 2018). Works under controlled conditions have successfully described reduced body condition indices after long-term exposures (eight months) to microplastics (Welden and Cowie, 2016a). Besides the size and position of balls, blockage of food passage did not seem to occur, as the occurrence of intestinal contents was high in most of the shrimps with balls. Some items of their ordinary diet include hard shells (e.g. bivalves, gastropods) or carapaces (e.g. other small crustaceans) amongst others (Cartes, 1994); hence, their digestive system might be able to cope with hard, resilient big items. Moreover, deep-sea, benthic organisms have evolved to be able to handle mixtures of edible and non-edible particles (Ogonowski et al., 2018). In fact, the presence of fibers was correlated with higher stomach fullness (volume), suggesting that there was no false satiation effect. The only negative correlation observed was between the fiber load and the gonadosomatic index (GSI) of shrimps off Barcelona (the sampling with the highest mean values of fiber ingestion). A recent meta-analysis of microplastics effects on aquatic organisms (Foley et al., 2018), reported that reproduction was the least commonly affected function, in contraposition to growth, consumption or survival. Given that the GSI is linked to energy reserves and trophic condition one would expect a certain delay between the ingestion and accumulation of fibers and a significant effect on GSI. It seems unlikely, with this rather fast increase in fiber load (over a two-month period), without a negative impact on stomach fullness, general body condition index or clear histopathological effects identified, that the presence of fibers on its own is the cause of reduced GSI. The fact that this correlation was only found in shrimps off Barcelona, after episodes of great rainfall and river discharge, could suggest the arrival of pulses of other pollutants (Koenig et al., 2013), with specific, unknown, mechanisms to produce a faster impact on reproduction (Kirby et al., 1999). In a parallel study performed in 2007, values of organic pollutants (PCBs, DDTs and PAHs) in sediments of the same area (off Barcelona) were considered low, and chemical exposure was regarded to have little influence on specific fish biomarkers (Solé et al., 2010). On the contrary, Sánchez-Avila et al. (2012), estimated a significant pollution risk (organic micropollutants) for sensitive mysid shrimps in coastal waters. Regarding heavy metals, a depocenter with high trace-metal contents (enrichment factors ranging between 1.2 and 10) was identified in front of the Llobregat river (Palanques et al., 2008). However, in the same work authors noted a high small-scale variability and that a significant dilution of metal concentration occurred deeper in the canyons. Since *A. antennatus* obtains macrofaunal prey from both outside and inside canyons (Cartes, 1994) a real relationship with heavy metals cannot be established without a specific study. Therefore, among other factors, possible negative effects or interactions with organic pollutants or heavy metals on shrimp's health could not be discarded.

5. Conclusions

Our findings demonstrate that *Aristeus antennatus* can experience acute episodes of plastic fiber accumulation in the digestive tract yet no consistent signs of a negative impact of fibers on shrimp's health condition were observed. Throughout the study, high values of fiber occurrence were found, especially in those areas where higher inputs of fibers into the environment are expected due to great anthropogenic pressure (high population density) and episodes of increased precipitation and river discharge. The variability in fiber ingestion observed in *A. antennatus* among locations and between years could suggest the potential for using this species as a monitor for fiber contamination in the deep-sea. Finally, but importantly, the results obtained demonstrate the need to improve our waste management policies, especially regarding anthropogenic fibers for which sources and pathways into the ocean are yet to be clearly identified.

Summary of the main findings

"High variability of fiber load in individuals of *Aristeus antennatus* from the NW Mediterranean coast sampled in different locations, years and seasons is described with no evidence of negative impact on health condition."

Declaration of competing interest

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

Acknowledgements

We are grateful to two anonymous reviewers for their comments and suggestions. We would like to thank Dr. Francesc Padrós for helping with the histological assessment of shrimps and for valuable discussions on the topic of shrimp's condition. This study was supported by the Spanish Ministry of Science, Innovation and Universities project "PLASMAR" (RTI2018-094806-B-100) and by the Catalan Department of Agriculture, Livestock, Fisheries and Food (European Maritime and Fisheries Fund (EMFF)) project "SOMPESCA" (ARP059/19/00003). We thank all fishermen from commercial fishing vessels involved in the "SOMPESCA" project. Carreras-Colom benefits from an FPU Ph.D. student grant from the Spanish Ministry of Science, Innovation and Universities (FPU16/03430).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114567.

References

Agència Catalana de l'Aigua (ACA) [WWW Document], 2019. http://aca-web.gencat.cat/sdim21 (accessed 9.20.04).

Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark *Galeus melastomus* Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ. Pollut. 223, 223–229. https://doi.org/10.1016/j.envpol.2017.01.015.

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1–10. https://doi.org/10.1016/j.marenvres.2016.01.005.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods (Plymouth).

Bagaev, A., Mizyuk, A., Khatmullina, L., Isachenko, I., Chubarenko, I., 2017. Anthropogenic fibres in the Baltic Sea water column: field data, laboratory and numerical testing of their motion. Sci. Total Environ. 599–600, 560–571. https://doi.org/10.1016/j.scitotenv.2017.04.185.

Beer, S., Garm, A., Huwer, B., Dierking, J., Nielsen, T.G., 2018. No increase in marine microplastic concentration over the last three decades – a case study from the Baltic Sea. Sci. Total Environ. 621, 1272–1279. https://doi.org/10.1016/j.scitotenv.2017.10.101.

Bell, S., 2006. Forensic Chemistry, first ed. Pearson Prentice Hall.

Bell, T.A., Lightner, D.V., 1988. A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society, Baton Rouge, Louisiana.

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179. https://doi.org/10.1021/es201811s.

Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer New York, New York, NY, p. 488. https://doi.org/10.1007/b97636, 2nd.

Canals, M., Puig, P., de Madron, X.D., Heussner, S., Palanques, A., Fabres, J., 2006. Flushing submarine canyons. Nature 444, 354–357. https://doi.org/10.1038/nature05271.

Carbonell, A., Grau, A., Laurence, V., Gómez, C., 2006. Ovary development of the red shrimp, *Aristeus antennatus* (risso, 1816) from the northwestern Mediterranean Sea. Crustaceana 79, 727–743. https://doi.org/10.1163/156854006778026807.

Carr, S.A., 2017. Sources and dispersive modes of micro-fibers in the environment. Integrated Environ. Assess. Manag. 13, 466–469. https://doi.org/10.1002/ieam.1916.

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182. https://doi.org/10.1016/j.watres.2016.01.002.

Carreras-Colom, E., Constenla, M., Soler-Membrives, A., Cartes, J.E., Baeza, M., Padrós, F., Carrassón, M., 2018. Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*. Mar. Pollut. Bull. 133, 44–52. https://doi.org/10.1016/j.marpolbul.2018.05.012.

Cartes, J.E., 1994. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea (western Mediterranean). Mar. Biol. 120, 639–648. https://doi.org/10.1007/BF00350085.

Cartes, J.E., López-Pérez, C., Carbonell, A., 2018. Condition and recruitment of *Aristeus antennatus* at great depths (to 2,300 m) in the Mediterranean: relationship with environmental factors. Fish. Oceanogr. 27, 114–126. https://doi.org/10.1111/fog.12237.

Collignon, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the north western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864. https://doi.org/10.1016/j.marpolbul.2012.01.011.

Compa, M., Ventero, A., Iglesias, M., Deudero, S., 2018. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar. Pollut. Bull. 128, 89–96. https://doi.org/10.1016/j.marpolbul.2018.01.009.

Constant, M., Kerhervé, P., Mino-Vercellio-Verollet, M., Dumontier, M., Sànchez Vidal, A., Canals, M., Heussner, S., 2019. Beached microplastics in the northwestern Mediterranean Sea. Mar. Pollut. Bull. 142, 263–273. https://doi.org/10.1016/j.marpolbul.2019.03.032.

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S.F., Narayanaswamy, B.E., 2019. Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976–2015), a study from the North East Atlantic. Environ. Pollut. 244, 503–512. https://doi.org/10.1016/j.envpol.2018.10.090.

Dantas, D.V., Barletta, M., da Costa, M.F., 2012. The seasonal and spatial patterns of ingestion of polyfilament nylon fragments by estuarine drums (Sciaenidae). Environ. Sci. Pollut. Res. 19, 600–606. https://doi.org/10.1007/s11356-011-0579-0.

Departament d'Agricultura, Ramaderia, Pesca i Alimentació (DARPA), 2019 [WWW Document]. http://agricultura.gencat.cat/ca/ambits/pesca/dar_estadistiques_pesca_subhastada (accessed 4.20.19).

de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the western Mediterranean Sea. Mar. Pollut. Bull. 140, 523–535. https://doi.org/10.1016/j.marpolbul.2019.01.053.

Demestre, M., 1995. Moult activity-related spawning success in the Mediterranean deep-water shrimp *Aristeus antennatus* (Decapoda:Dendrobranchiata). Mar. Ecol. Prog. Ser. 127, 57–64. https://doi.org/10.3354/meps127057.

Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., Vethaak, A.D., 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, linnaeus 1758) from coastal waters of the southern North Sea and channel area. Mar. Pollut. Bull. 98, 179–187. https://doi.org/10.1016/j.marpolbul.2015.06.051.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., Tassin, B., 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ. Pollut. 221, 453–458. https://doi.org/10.1016/j.envpol.2016.12.013.

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PloS One 9, e111913. https://doi.org/10.1371/journal.pone.0111913.

Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631–632, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046.

Font, J., Salat, J., Tintoré, J., 1988. Permanent features in the circulation of the Catalan Sea. In: Minas, H.J., Nival, P. (Eds.), Oceanologica Acta. Oceanographie Pelagique Méditerranéene.

Gago, J., Carretero, O., Filgueiras, A.V., Viñas, L., 2018. Synthetic microfibers in the marine environment: a review on their occurrence in seawater and sediments. Mar. Pollut. Bull. 127, 365–376. https://doi.org/10.1016/j.marpolbul.2017.11.070.

Galgani, F., Jaunet, S., His, E., 1995. Distribution and Abundance of Debris on the Continental Shelf of the North-Western Mediterranean Sea, vol. 31, pp. 713–717.

Galimany, E., Marco-Herrero, E., Soto, S., Recasens, L., Lombarte, A., Lleonart, J., Abelló, P., Ramón, M., 2019. Benthic marine litter in shallow fishing grounds in the NW Mediterranean Sea. Waste Manag. 95, 620–627. https://doi.org/10.1016/j.wasman.2019.07.004.

García-Rivera, S., Lizaso, J.L.S., Millán, J.M.B., 2018. Spatial and temporal trends of marine litter in the Spanish Mediterranean seafloor. Mar. Pollut. Bull. 137, 252–261. https://doi.org/10.1016/j.marpolbul.2018.09.051.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782 https://doi.org/10.1126/sciadv.1700782.

Gündoğdu, S., Çevik, C., Ayat, B., Aydoğan, B., Karaca, S., 2018. How microplastics quantities increase with flood events? An example from Mersin Bay NE Levantine coast of Turkey. Environ. Pollut. 239, 342–350. https://doi.org/10.1016/j.envpol.2018.04.042.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297.

Institut d'Estadística de Catalunya (IDESCAT), 2019 [WWW Document]. http://www.idescat.cat/serveis/consultes (accessed 4.20.19).

Imhof, H.K., Sigl, R., Brauer, E., Feyl, S., Giesemann, P., Klink, S., Leupolz, K., Löder, M.G.J., Löschel, L.A., Missun, J., Muszynski, S., Ramsperger, A.F.R.M., Schrank, I., Speck, S., Steibl, S., Trotter, B., Winter, I., Laforsch, C., 2017. Spatial and temporal variation of macro-, meso- and microplastic abundance on a remote coral island of the Maldives. Indian Ocean. Mar. Pollut. Bull. 116, 340–347. https://doi.org/10.1016/j.marpolbul.2017.01.010.
Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10, 417. https://doi.org/10.1038/s41467-019-08316-9.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80- 347, 768–771. https://doi.org/10.1126/science.1260352.
Kirby, M.F., Matthiessen, P., Neall, P., Tylor, T., Allchin, C.R., Kelly, C.A., Maxwell, D.L., Thain, J.E., 1999. Hepatic EROD activity in flounder (*Platichthys flesus*) as an indicator of contaminant exposure in English estuaries. Mar. Pollut. Bull. 38, 676–686. https://doi.org/10.1016/S0025-326X(98)00201-X.
Koenig, S., Fernández, P., Company, J.B., Huertas, D., Solé, M., 2013. Are deep-sea organisms dwelling within a submarine canyon more at risk from anthropogenic contamination than those from the adjacent open slope? A case study of Blanes canyon (NW Mediterranean). Prog. Oceanogr. 118, 249–259. https://doi.org/10.1016/j.pocean.2013.07.016.
Ladewig, S.M., Bao, S., Chow, A.T., 2015. Natural fibers: a missing link to chemical pollution dispersion in aquatic environments. Environ. Sci. Technol. 49, 12609–12610. https://doi.org/10.1021/acs.est.5b04754.
Le Cren, E.D., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J. Anim. Ecol. 20, 201. https://doi.org/10.2307/1540.
Lechner, A., Ramler, D., 2015. The discharge of certain amounts of industrial microplastic from a production plant into the River Danube is permitted by the Austrian legislation. Environ. Pollut. 200, 159–160. https://doi.org/10.1016/j.envpol.2015.02.019.
Lima, A.R.A., Costa, M.F., Barletta, M., 2014. Distribution patterns of microplastics within the plankton of a tropical estuary. Environ. Res. 132, 146–155. https://doi.org/10.1016/j.envres.2014.03.031.
Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G.
Markit, I., 2016. Acrylic and Modacrylic Fibers - Chemical Economics Handbook.
Menges, F., 2019. Spectragryph - Optical Spectroscopy Software.
Servei Meteorològic de Catalunya (Meteocat), 2019 [WWW Document]. www.meteo.cat/wpweb/climatologia/serveis-i-dades-climatiques (accessed 4.20.19).
Miquel, J.C., Fowler, S.W., La Rosa, J., Buat-Menard, P., 1994. Dynamics of the downward flux of particles and carbon in the open northwestern Mediterranean Sea. Deep-Sea Res. Part I Oceanogr. Res. Pap. 41, 243–261. https://doi.org/10.1016/0967-0637(94)90002-7.
Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50, 5800–5808. https://doi.org/10.1021/acs.est.5b05416.
Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032.
Nadal, M.A., Alomar, C., Deudero, S., 2016. High levels of microplastic ingestion by the semipelagic fish bogue Boops boops (L.) around the Balearic Islands. Environ. Pollut. 214, 517–523. https://doi.org/10.1016/j.envpol.2016.04.054.
Ogonowski, M., Gerdes, Z., Gorokhova, E., 2018. What we know and what we think we know about microplastic effects – a critical perspective. Curr. Opin. Environ. Sci. Health 1, 41–46. https://doi.org/10.1016/j.coesh.2017.09.001.
Palanques, A., Masqué, P., Puig, P., Sanchez-Cabeza, J.A., Frignani, M., Alvisi, F., 2008. Anthropogenic trace metals in the sedimentary record of the Llobregat continental shelf and adjacent Foix Submarine Canyon (northwestern Mediterranean). Mar. Geol. 248, 213–227. https://doi.org/10.1016/j.margeo.2007.11.001.
Pedrotti, M.L., Petit, S., Elineau, A., Bruzaud, S., Crebassa, J.C., Dumontet, B., Martí, E., Gorsky, G., Cózar, A., 2016. Changes in the floating plastic pollution of the mediterranean sea in relation to the distance to land. PloS One 11, 1–14. https://doi.org/10.1371/journal.pone.0161581.
Ramirez-Llodra, E., De Mol, B., Company, J.B., Coll, M., Sardà, F., 2013. Effects of natural and anthropogenic processes in the distribution of marine litter in the deep Mediterranean Sea. Prog. Oceanogr. 118, 273–287. https://doi.org/10.1016/j.pocean.2013.07.027.
Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711. https://doi.org/10.1002/etc.4371.
Rodriguez-Seijo, A., Lourenço, J., Rocha-Santos, T.A.P., da Costa, J., Duarte, A.C., Vala, H., Pereira, R., 2017. Histopathological and molecular effects of microplastics in *Eisenia andrei* Bouché. Environ. Pollut. 220, 495–503. https://doi.org/10.1016/j.envpol.2016.09.092.
Ruiz-Orejón, L.F., Sardá, R., Ramis-Pujol, J., 2016. Floating plastic debris in the central and western Mediterranean Sea. Mar. Environ. Res. 120, 136–144. https://doi.org/10.1016/j.marenvres.2016.08.001.
Rumolo, P., Cartes, J.E., Fanelli, E., Papiol, V., Sprovieri, M., Mirto, S., Gherardi, S., Bonanno, A., 2015. Seasonal variations in the source of sea bottom organic matter off Catalonia coasts (western Mediterranean): links with hydrography and biological response. J. Oceanogr. 71, 325–343. https://doi.org/10.1007/s10872-015-0291-7.
Saborowski, R., Paulischkis, E., Gutow, L., 2019. How to get rid of ingested microplastic fibers? A straightforward approach of the Atlantic ditch shrimp *Palaemon varians*. Environ. Pollut. 254, 113068. https://doi.org/10.1016/j.envpol.2019.113068.
Confedereración Hidrográfica del Ebro (SAIH), 2019 [WWW Document], n.d. http://www.saihebro.com/saihebro/index.php?url /autoservicio/inicio (accessed 4.20.19).
Salvador Cesa, F., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. Sci. Total Environ. 598, 1116–1129. https://doi.org/10.1016/j.scitotenv.2017.04.172.
Sánchez-Avila, J., Tauler, R., Lacorte, S., 2012. Organic micropollutants in coastal waters from NW Mediterranean Sea: sources distribution and potential risk. Environ. Int. 46, 50–62. https://doi.org/10.1016/j.envint.2012.04.013.
Sanchez-Vidal, A., Higueras, M., Martí, E., Liquete, C., Calafat, A., Kerhervé, P., Canals, M., 2013. Riverine transport of terrestrial organic matter to the North Catalan margin, NW Mediterranean Sea. Prog. Oceanogr. 118, 71–80. https://doi.org/10.1016/j.pocean.2013.07.020.
Sanchez-Vidal, A., Thompson, R.C., Canals, M., de Haan, W.P., 2018. The imprint of microfibres in southern European deep seas. PloS One 13, e0207033. https://doi.org/10.1371/journal.pone.0207033.
Schmidt, N., Thibault, D., Galgani, F., Paluselli, A., Sempéré, R., 2018. Occurrence of microplastics in surface waters of the Gulf of lion (NW Mediterranean Sea). Prog. Oceanogr. 163, 214–220. https://doi.org/10.1016/j.pocean.2017.11.010.
Siegfried, M., Koelmans, A.A., Besseling, E., Kroeze, C., 2017. Export of microplastics from land to sea. A modelling approach. Water Res. 127, 249–257. https://doi.org/10.1016/j.watres.2017.10.011.
Solé, M., Baena, M., Arnau, S., Carrasson, M., Maynou, F., Cartes, J.E., 2010. Muscular cholinesterase activities and lipid peroxidation levels as biomarkers in several Mediterranean marine fish species and their relationship with ecological variables. Environ. Int. 36, 202–211. https://doi.org/10.1016/j.envint.2009.11.008.
Torre, M., Digka, N., Anastasopoulou, A., Tsangaris, C., Mytilineou, C., 2016. Anthropogenic microfibres pollution in marine biota. A new and simple methodology to minimize airborne contamination. Mar. Pollut. Bull. 113, 55–61. https://doi.org/10.1016/j.marpolbul.2016.07.050.
Tubau, X., Canals, M., Lastras, G., Rayo, X., Rivera, J., Amblas, D., 2015. Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: the role of hydrodynamic processes. Prog. Oceanogr. 134, 379–403. https://doi.org/10.1016/j.pocean.2015.03.013.
UNEP, 2009. Marine Litter: a Global Challenge (Nairobi).
UNEP/MAP, 2015. Marine Litter Assessment in the Mediterranean (Athens, Greece).
Von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335. https://doi.org/10.1021/es302332w.
Wagner, M., Lambert, S., 2018. Freshwater Microplastics, the Handbook of Environmental Chemistry. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-61615-5.
Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., Hughes, K.A., 2017. Microplastics in the Antarctic marine system: an emerging area of research. Sci. Total Environ. 598, 220–227. https://doi.org/10.1016/j.scitotenv.2017.03.283.
Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. Environ. Sci. Technol. 49, 14597–14604. https://doi.org/10.1021/acs.est.5b04026.
Welden, N.A.C., Cowie, P.R., 2016a. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ. Pollut. 218, 895–900. https://doi.org/10.1016/j.envpol.2016.08.020.
Welden, N.A.C., Cowie, P.R., 2016b. Environment and gut morphology influence microplastic retention in langoustine, *Nephrops norvegicus*. Environ. Pollut. 214, 859–865. https://doi.org/10.1016/j.envpol.2016.03.067.
Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1 https://doi.org/10.1098/rsos.140317, 140317–140317.

Supplementary material

A closer look at anthropogenic fiber ingestion in *Aristeus antennatus* in the NW Mediterranean Sea: differences among years and locations and impact on health condition

Ester Carreras-Colom[a], María Constenla[a], Anna Soler-Membrives[a], Joan E. Cartes[b], Mireia Baeza[c], Maite Carrassón[a,*]

[a] *Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain*
[b] *Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain*
[c] *Departament de Química, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain*

[*] *Corresponding author, e-mail: Maite.Carrassón@uab.cat*

Table S1. Categorization of fibers encountered in the digestive system of *Aristeus antennatus* according to visual characteristics (general aspect) and results of the identification of 119 anthropogenic fibers (2.9% of the total) by means of FTIR (percentage of each polymer identified in %). Polymers identified Acr. = Acrylic; Cel. = Cellulose; PA = Polyamide; PET = Polyethylene terephthalate; PP = polypropylene.

Category	Description	Polymer identified (%)				
		Acr.	Cel.	PA	PET	PP
A	• Uniform diameter and round cross-section • Sometimes with wide molten or frayed ends • Generally smooth surface texture. Granular backbone texture. Pilling or fraying surface when damaged • Mostly transparent, yellowed or brownish	0	0	**70.8**	16.7	12.5
B	• Mostly uniform diameter (sometimes with molten bends) and round cross-section • Clean ends, sometimes molten • Smooth surface texture. Refringent. Usually with delustrant agents visible as a bubbly backbone texture • Generally transparent or bright colored	8.1	0	0	**81.1**	10.8
C	• Non-uniform diameter, flat or film-like • Diagonal-cut ends • Wrinkled surface with angular edges. Sometimes fraying surface • Mostly transparent, blue or black, usually non-uniform	0	**100**	0	0	0
D	• Non-uniform diameter with dumbbell cross-section • Usually with fraying ends • Smooth and homogeneous surface and backbone texture. • Mostly transparent or bright colors	**80**	0	0	14.3	5.7
E	• Non-uniform diameter with almost round-section • Generally clean ends • Wrinkled with smoothed or round edges • Mostly smooth texture (no fraying) • Mostly with dark colors	10	20	0	**70**	0

Table S2. Descriptive parameters for fiber occurrence and load in individuals of *Aristeus antennatus* from three localities along the Catalan Coast (off Costa Brava, off Barcelona city, and off Ebro Delta) for spring and summer samplings in 2018 and according to their location in the digestive tract (stomach or intestine). Significant differences are indicated with superscripts as follows: seasonal differences (within the same locality) are denoted with numbers and spatial differences (within the same season) are indicated with letters (low case letters for spring and capital letters for summer). Absence of letters or numbers indicates no differences were found. Mean values ± standard deviation are given except for occurrence values (in percentage). FO: fiber occurrence; TA: total abundance of fiber per individual; TL: total length of fibers per individual; TLs: total length of synthetic fibers per individual; BO: ball occurrence; BA: estimated area per ball; BD: estimated density per ball.

	Costa Brava		Barcelona city		Ebro Delta	
	spring	summer	spring	summer	spring	summer
STOMACH						
FO (%)	68.2	60.0 ^{A}	80.8	94.4 ^{B}	100.0	76.5 ^{AB}
TA (n fibers/ind)	1.82 ± 2.11 ^{a}	10.0 ± 29.59 ^{A}	7.48 ± 17.90 ^{1,b}	157.33 ± 129.45 ^{2,B}	7.89 ± 4.08 ^{c}	16.41 ± 23.49 ^{A}
TL (mm of fibers/ind)	6.44 ± 7.05 ^{a}	33.34 ± 71.32 ^{A}	32.36 ± 54.22 ^{1,b}	888.73 ± 637.59 ^{2,B}	32.42 ± 18.79 ^{b}	85.63 ± 109.28 ^{A}
BO (%)	4.5 ^{1,a}	35.0 ^{2,A}	34.8 ^{1,b}	88.9 ^{2,B}	77.8 ^{1,c}	52.9 ^{1,A}
BA (mm2/ball)	1.02 ^{1,a*}	1.73 ± 0.98 ^{2,A}	2.06 ± 0.26 ^{1,b}	18.53 ± 19.76 ^{2,B}	1.48 ± 0.66 ^{c}	2.63 ± 1.65 ^{A}
BD (mm/mm2·ball)	17.37 ^{1,a*}	36.55 ± 26.20 ^{2,A}	32.36 ± 41.88 ^{1,b}	62.46 ± 34.39 ^{2,B}	28.98 ± 20.21 ^{c}	43.54 ± 30.24 ^{A}
INTESTINE						
FO (%)	50.0 ^{1}	5.0 ^{2,A}	30.4 ^{1}	88.9 ^{2,B}	66.7 ^{1}	17.6 ^{2,A}
TA (n fibers/ind)	0.91 ± 1.11 ^{1}	0.05 ± 0.22 ^{2,A}	0.65 ± 1.23 ^{1}	6.72 ± 5.05 ^{2,B}	1.11 ± 0.93 ^{1}	0.24 ± 0.56 ^{2,A}
TL (mm of fibers/ind)	2.19 ± 2.86 ^{1}	0.27 ± 1.20 ^{2,A}	2.62 ± 8.06 ^{1}	26.32 ± 21.61 ^{2,B}	1.95 ± 1.62	0.97 ± 2.20 ^{A}
TOTAL						
FO (%)	77.3	65.0 ^{A}	95.7	94.4 ^{B}	100	82.4 ^{AB}
TA (n fibers/ind)	2.73 ± 2.69 ^{a}	10.05 ± 29.58 ^{A}	8.13 ± 17.78 ^{1,b}	164.06 ± 130.47 ^{2,B}	9.00 ± 4.58 ^{b}	16.65 ± 23.54 ^{A}
TL (mm of fibers/ind)	8.63 ± 7.74 ^{a}	33.61 ± 71.19 ^{A}	34.98 ± 53.60 ^{1,b}	915.05 ± 640.56 ^{2,B}	34.37 ± 20.09 ^{b}	86.60 ± 109.19 ^{A}
TLs (mm of plastic/individual)	7.07 ± 6.66 ^{a}	32.03 ± 70.99 ^{A}	34.65 ± 53.12 ^{1,b}	899.34 ± 630.04 ^{2,B}	33.38 ± 19.88 ^{b}	73.72 ± 104.23 ^{A}

* Only one ball found.

Table S3. Descriptive parameters for fiber occurrence and load in individuals in individuals of *Aristeus antennatus* off Barcelona city (just in front of the Besòs River or just south of the Llobregat River) for spring and summer samplings in 2007, 2017 and 2018, and according to their location in the digestive tract (stomach or intestine). Significant differences are indicated with superscripts as follows: seasonal differences (within the same year and locality) are denoted with numbers and temporal differences (within the same season and locality) are indicated with letters (low case letters for spring and capital letters for summer). Mean values ± standard deviation are given except for occurrence values (in percentage). FO: fiber occurrence; TA: total abundance of fibers per individual; TL: total fiber length; TLs: total length of synthetic fibers per individual; BO: ball occurrence; BA: ball area; BD: ball density. More details on each sampling station can be found in Table 1.

	Besòs			Llobregat		
	2007		2017	2007	2018	
	spring (B2B)	summer (B3B)	spring (P0B)	summer (B3V)	spring (P1V)	summer (P2V)
STOMACH						
FO (%)	93.8 ^{1,a}	71.0 ^{1}	56.0 ^{b}	80.0 ^{A}	91.3 ^{1}	94.4 ^{1,A}
TA (n fibers/ind)	6.38 ± 7.54 ^{1,a}	15.32 ± 26.55 ^{1}	2.60 ± 4.85 ^{b}	5.10 ± 7.85 ^{A}	7.48 ± 17.90 ^{1}	157.33 ± 129.45 ^{2,B}
TL (mm of fibers/ind)	42.87 ± 51.89 ^{1,a}	61.75 ± 99.05 ^{1}	18.74 ± 37.06 ^{b}	29.80 ± 59.93 ^{A}	32.36 ± 54.22 ^{1}	888.73 ± 637.59 ^{2,B}
BO (%)	50.0 ^{1,a}	38.7 ^{1}	16.0 ^{b}	25.0 ^{A}	34.8 ^{1}	88.9 ^{2,B}
BA (mm2/ball)	2.97 ± 1.88 ^{1,a}	3.60 ± 3.12 ^{1}	4.87 ± 2.88 ^{a}	3.09 ± 3.07 ^{A}	2.06 ± 0.26 ^{1}	18.53 ± 19.76 ^{2,B}
BD (mm/mm2·ball)	27.58 ± 17.70 ^{1,a}	50.34 ± 28.62 ^{1}	15.44 ± 4.23 ^{b}	33.45 ± 17.07 ^{A}	32.36 ± 41.88 ^{1}	62.46 ± 34.39 ^{2,B}
INTESTINE						
FO (%)	68.8 ^{1,a}	32.3 ^{2}	48.0 ^{a}	35.0 ^{A}	30.4 ^{1}	88.9 ^{2,B}
TA (n fibers/ind)	1.50 ± 1.59 ^{1,a}	0.55 ± 1.09 ^{2}	1.20 ± 2.10 ^{a}	0.50 ± 0.76 ^{A}	0.65 ± 1.23 ^{1}	6.72 ± 5.05 ^{2,B}
TL (mm of fibers/ind)	5.67 ± 8.12 ^{1,a}	2.03 ± 5.49 ^{2}	4.34 ± 7.29 ^{a}	2.25 ± 3.86 ^{A}	2.62 ± 8.06 ^{1}	26.32 ± 21.61 ^{2,B}
TOTAL						
FO (%)	100.0 ^{1,a}	74.2 ^{2}	72.0 ^{b}	85.0 ^{A}	95.7 ^{1}	94.4 ^{1,A}
TA (n fibers/ind)	7.88 ± 7.86 ^{1,a}	15.87 ± 26.51 ^{1}	3.80 ± 5.31 ^{b}	5.60 ± 8.24 ^{A}	8.13 ± 17.78 ^{1}	164.06 ± 130.47 ^{2,B}
TL (mm of fibers/ind)	48.54 ± 53.19 ^{1,a}	63.78 ± 98.23 ^{1}	23.08 ± 37.51 ^{b}	32.05 ± 60.39 ^{A}	34.98 ± 53.60 ^{1}	915.05 ± 640.56 ^{2,B}
TLs (mm of synthetic fibers/individual)	47.65 ± 53.78 ^{1,a}	62.37 ± 95.42 ^{1}	22.45 ± 37.39 ^{b}	31.15 ± 60.52 ^{A}	34.65 ± 53.12 ^{1}	899.34 ± 630.04 ^{2,B}

Table S4. Summary of biological parameters, including size and body condition indices, for each sampling station. Mean values ± SD are given.

Code	***n***	**CL (mm)**	**Kn**	**HSI**	**GSI**
B2B	16	35.0-47.4	0,957 ± 0,070	6,29 ± 1,67	0,40 ± 0,20
B3B	31	22.9-46.6	0,957 ± 0,064	4,31 ± 1,97	3,11 ± 3,14
B3V	20	22.5-44.7	0,978 ± 0,074	2,96 ± 1,49	3,43 ± 2,34
P0B	25	33.8-42.5	1,051 ± 0,073	6,48 ± 1,54	0,39 ± 0,23
P1G	22	26.8-31.2	1,013 ± 0,100	7,36 ± 1,18	0,38 ± 0,22
P1V	23	26.8-35.6	1,019 ± 0,066	8,02 ± 2,31	1,63 ± 1,00
P1D	9	33.4-37.3	1,026 ± 0,066	5,91 ± 1,73	0,34 ± 0,22
P2G	20	25.9-31.9	1,023 ± 0,088	5,72 ± 1,80	3,73 ± 2,12
P2V	18	31.1-38.3	0,984 ± 0,050	5,54 ± 1,24	6,67 ± 2,34
P2D	17	33.1-40.7	0,994 ± 0,062	5,39 ± 1,05	1,26 ± 0,93

Table S5. Studies conducted in the NW Mediterranean Sea reporting environmental concentrations of microplastics or marine litter.

Location	Year	Size range (mm)	Analysis Method	Depth range (m)	Particle concentrations average (± SD)	units	Source	Notes
Blanes	2018	0.16-22.4	Digestive content screening	396-641	6.399 21.12	fibers ·ind^{-1} mm ·ind^{-1}	Our study	
Barcelona	2007	0.35-37.7	Digestive content screening	790	9.78 48.12	fibers ·ind^{-1} mm ·ind^{-1}	Our study	
Barcelona	2017-2018	0.21-37.3	Digestive content screening	572-785	58.66 324.37	fibers ·ind^{-1} mm ·ind^{-1}	Our study	
Delta	2018	0.62-37.9	Digestive content screening	425-551	12.83 60.49	fibers ·ind^{-1} mm ·ind^{-1}	Our study	
Surface waters								
Blanes [a]	2011-2012	0.33-5	Manta trawl (335 µm)	0	80000-160000	items · km^{-2}	[1]	No exact values given for the specific area of Blanes.
Blanes [a]	2015	0.33-5	Manta trawl (335 µm)	0	0.497 0.080	items · m^{-2} mg·m^{-2}	[2]	Fibers were not counted.
Barcelona [b]	2011-2012	0.33-5	Manta trawl (335 µm)	0	>320000	items · km^{-2}	[1]	No exact values given for the specific area of Barcelona.
Barcelona [b]	2015	0.33-5	Manta trawl (335 µm)	0	0.110 0.023	items · m^{-2} mg·m^{-2}	[2]	Fibers were not counted.
Catalan coast	2015	0.33-5	Manta trawl (335 µm)	0	0.183 ± 0.158 0.025 ± 0.025	items · m^{-2} mg·m^{-2}	[2]	Fibers were not counted.
Balearic Basin	2013	0.2-1000	Neuston net (200 µm)	0.2	549.6	g · km^{-2}	[3]	Broader area than our study area.
Gulf of Lion	2010	0.33-5	Manta trawl (335 µm)	0	0.06	mg·m^{-2}	[4]	Close, yet not our area of study.
Gulf of Lion	2015	0.2-5	WP2 net (200 µm)	0	0.23 ± 0.20	items · m^{-3}	[5]	Close, yet not our area of study
W Mediterranean	2010	0.33-5	Manta trawl (335 µm)	0	0.116 2.02	items · m^{-2} mg·m^{-2}	[4]	Much broader area than our study area.
All Mediterranean	2011-2012	0.33-5	Manta trawl (335 µm)	0	129682 62.211	items · km^{-2} mg·km^{-2}	[1]	Much broader area than our study area.
All Mediterranean	2011-2012	>5	Manta trawl (335 µm)	0	5700 12000	items · km^{-2} mg·km^{-2}	[1]	Much broader area than our study area.

Sardinian-Balearic transect	2013-2016	>200	Visual survey	0	2.5	items · km^{-2}	[6]	Close, yet not our study area. Macrolitter, including non-plastic items, considered
Coastal sediments / Beach sand								
Barcelona [b]	2015-2017	<5*	Beach sediment	0	148	items · kg^{-1}	[7]	Far from deep-sea areas.
Ebro Delta [c]	2017	<0.05->0.3	Beach sediment	0-5	422 ± 119	items · kg^{-1}	[8]	Far from deep-sea areas.
Ebro Delta [c]	2017	<0.05->0.3	Riverbed sediment	0-5	2052 ± 746	items · kg^{-1}	[8]	Far from deep-sea areas.
Cap Croisette (Gulf of Lion)	2016	0.063 – >5	Beach sediment	0	4,654	items · m^{-2}	[9]	Far from deep-sea areas.
Balearic Islands	2013	0.063 – 5	Subtidal sediment	8-10	0.27	items · g^{-1}	[10]	Far from deep-sea areas.
Seafloor								
Blanes [a]	2009-2012	3-8	Deep-sea sediment	67-2222	8222 ± 3,700	fibers · m^{-2}	[11]	Similar approach (man-made fibers counted).
Blanes [a]	1994-1996	macro	Trawl composition	40-1600	~1,600	items · km^{-2}	[12]	Macrolitter considered, yet only plastic items.
Blanes [a]	1999-2011	macro	Trawl composition	35-4500	31.1 0.7-1.2	items · ha^{-1} kg · ha^{-1}	[13]	Macrolitter, including non-plastic items, considered
Blanes [a]	2009	macro	Trawl composition	900-2700	0.02-3264.6	kg · km^{-2}	[14]	Macrolitter, including non-plastic items, considered
Blanes [a]	2015	macro	Visual survey	860-1509	1559	items · km^{-2}	[15]	Macrolitter, including non-plastic items, considered
Barcelona [b]	1993-1994	macro	Trawl composition	-	1762.6	items · km^{-2}	[16]	Macrolitter, including non-plastic items, considered
Cap de Creus	2009	20-500	Trawl composition	40-80	60.03	items · ha^{-1}	[17]	Macrolitter, including non-plastic items, considered
Catalan coast	2007-2017	>20	Trawl composition	0-800	~3,1	kg · km^{-2}	[18]	Macrolitter, including non-plastic items, considered
French coast	1992-1998	macro	Trawl composition	-	19.35	items · ha^{-1}	[19]	Macrolitter, including non-plastic items, considered

[a] Equivalent to our Costa Brava sampling location
[b] Equivalent to our Barcelona sampling location
[c] Equivalent to our Delta sampling location

[1] F. Faure, C. Saini, G. Potter, F. Galgani, L.F. de Alencastro, P. Hagmann, An evaluation of surface micro- and mesoplastic pollution in pelagic ecosystems of the Western Mediterranean Sea, Environ. Sci. Pollut. Res. 22 (2015) 12190–12197. https://doi.org/10.1007/s11356-015-4453-3.
[2] W.P. de Haan, A. Sanchez-Vidal, M. Canals, Floating microplastics and aggregate formation in the Western Mediterranean Sea, Mar. Pollut. Bull. 140 (2019) 523–535. https://doi.org/10.1016/j.marpolbul.2019.01.053.
[3] A. Cózar, M. Sanz-Martín, E. Martí, J.I. González-Gordillo, B. Ubeda, J. Á.gálvez, X. Irigoien, C.M. Duarte, Plastic accumulation in the mediterranean sea, PLoS One. 10 (2015) 1–12. https://doi.org/10.1371/journal.pone.0121762.
[4] A. Collignon, J.-H. Hecq, F. Glagani, P. Voisin, F. Collard, A. Goffart, Neustonic microplastic and zooplankton in the North Western Mediterranean Sea, Mar. Pollut. Bull. 64 (2012) 861–864. https://doi.org/10.1016/j.marpolbul.2012.01.011.
[5] C. Lefebvre, C. Saraux, O. Heitz, A. Nowaczyk, D. Bonnet, Microplastics FTIR characterisation and distribution in the water column and digestive tracts of small pelagic fish in the Gulf of Lions, Mar. Pollut. Bull. 142 (2019) 510–519. https://doi.org/10.1016/j.marpolbul.2019.03.025.
[6] A. Arcangeli, I. Campana, D. Angeletti, F. Atzori, M. Azzolin, L. Carosso, V. Di Miccoli, A. Giacoletti, M. Gregorietti, C. Luperini, M. Paraboschi, G. Pellegrino, M. Ramazio, G. Sarà, R. Crosti, Amount, composition, and spatial distribution of floating macro litter along fixed trans-border transects in the Mediterranean basin, Mar. Pollut. Bull. 129 (2018) 545–554. https://doi.org/10.1016/j.marpolbul.2017.10.028.
[7] F.A.E. Lots, P. Behrens, M.G. Vijver, A.A. Horton, T. Bosker, A large-scale investigation of microplastic contamination : Abundance and characteristics of microplastics in European beach sediment, Mar. Pollut. Bull. 123 (2017) 219–226. https://doi.org/10.1016/j.marpolbul.2017.08.057.
[8] L. Simon-Sánchez, M. Grelaud, J. Garcia-Orellana, P. Ziveri, River Deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean), Sci. Total Environ. 687 (2019) 1186–1196. https://doi.org/10.1016/j.scitotenv.2019.06.168.
[9] M. Constant, P. Kerhervé, M. Mino-Vercellio-Verollet, M. Dumontier, A. Sànchez Vidal, M. Canals, S. Heussner, Beached microplastics in the Northwestern Mediterranean Sea, Mar. Pollut. Bull. 142 (2019) 263–273. https://doi.org/10.1016/j.marpolbul.2019.03.032.
[10] C. Alomar, F. Estarellas, S. Deudero, Microplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size, Mar. Environ. Res. 115 (2016) 1–10. https://doi.org/10.1016/j.marenvres.2016.01.005.
[11] A. Sanchez-Vidal, R.C. Thompson, M. Canals, W.P. de Haan, The imprint of microfibres in southern European deep seas, PLoS One. 13 (2018) e0207033. https://doi.org/10.1371/journal.pone.0207033.
[12] F. Galgani, A. Souplet, Y. Cadiou, Accumulation of debris on the deep sea floor off the French Mediterranean coast, Mar. Ecol. Ser. 142 (1996) 225–234. https://doi.org/10.3354/meps142225.
[13] C.K. Pham, E. Ramirez-Llodra, C.H.S. Alt, T. Amaro, M. Bergmann, M. Canals, J.B. Company, J. Davies, G. Duineveld, F. Galgani, K.L. Howell, V.A.I. Huvenne, E. Isidro, D.O.B. Jones, G. Lastras, T. Morato, J.N. Gomes-Pereira, A. Purser, H. Stewart, I. Tojeira, T. Morato, X. Tubau, D. Van Rooij, P.A. Tyler, Marine Litter Distribution and Density in European Seas, from the Shelves to Deep Basins, PLoS One. 9 (2014). https://doi.org/10.1371/journal.pone.0095839.
[14] E. Ramirez-Llodra, B. De Mol, J.B. Company, M. Coll, F. Sardà, Effects of natural and anthropogenic processes in the distribution of marine litter in the deep Mediterranean Sea, Prog. Oceanogr. 118 (2013) 273–287. https://doi.org/10.1016/j.pocean.2013.07.027.
[15] X. Tubau, M. Canals, G. Lastras, X. Rayo, J. Rivera, D. Amblas, Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: The role of hydrodynamic processes, Prog. Oceanogr. 134 (2015) 379–403. https://doi.org/10.1016/j.pocean.2015.03.013.
[16] F. Galgani, S. Jaunet, A. Campillo, X. Guenegen, E. His, Distribution and abundance of debris on the continental shelf of the north-western Mediterranean Sea, Mar. Pollut. Bull. 30 (1995) 713–717. https://doi.org/10.1016/0025-326X(95)00055-R.
[17] P. Sánchez, M. Masó, R. Sáez, S. De Juan, A. Muntadas, M. Demestre, Baseline study of the distribution of marine debris on soft-bottom habitats associated with trawling grounds in the northern Mediterranean, Sci. Mar. 77 (2013) 247–255. https://doi.org/10.3989/scimar03702.10A.
[18] S. García-Rivera, J.L.S. Lizaso, J.M.B. Millán, Spatial and temporal trends of marine litter in the Spanish Mediterranean seafloor, Mar. Pollut. Bull. 137 (2018) 252–261. https://doi.org/10.1016/j.marpolbul.2018.09.051.
[19] F. Galgani, J.P. Leaute, P. Moguedet, A. Souplet, Y. Verin, A. Carpentier, Ò. Houmeau, J. Vilar, Litter on the Sea Floor Along European Coasts, Mar. Pollut. Bull. 40 (2000) 516–527. https://doi.org/10.1016/S0025-326X(99)00234-9.

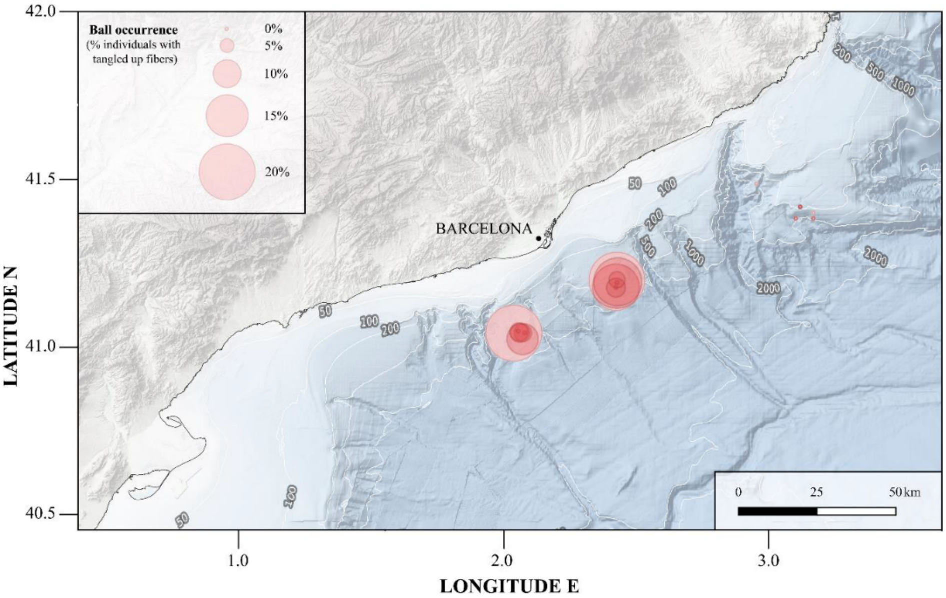


Fig. S1. Map of the study area showing the occurrence of balls (BO in %) in stomachs of *Aristeus antennatus* captured along the Catalan coast during 1988-1989 in a monthly sampling (n=768 specimens analyzed for diet studies). BO is calculated as the percentage of individuals with balls (tangled up fibers) over the total of individuals analyzed per each sampling. Largest circles represent BO = 20%. Differences in color intensity compared to the legend presented are due to the superposition of values for individuals from different samplings.

S9

CHAPTER 5 Health status assessment in *Nephrops norvegicus* (Linnaeus, 1758) from the NW Mediterranean Sea in relation to plastic contamination and trace metals

5 Health status assessment in *Nephrops norvegicus* (Linnaeus, 1758) from the NW Mediterranean Sea in relation to plastic contamination and trace metals

Ester Carreras-Colom[a], Joan E. Cartes[b], Oriol Rodríguez-Romeu[a], Maria Constenla[a], Francesc Padrós[a] Montserrat Solé[b], Michaël Grelaud[c], Cristina Palet[d], Anna Soler-Membrives[a], Maite Carrassón[a]*

[a] *Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain*
[b] *Departament de Recursos Naturals, Institut de les Ciències del Mar (ICM-CSIC), 08003 Barcelona, Spain.*
[c] *Institute of Environmental Science and Techonology (ICTA-UAB), Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain*
[d] *Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain*

*Corresponding author: maite.carrason@uab.cat

SUMMARY

The impacts of human activities on ecosystems and organisms are multifactorial but pose a significant challenge for a proper health assessment. This study aims to provide a multidisciplinary health risk assessment for the population of *Nephrops norvegicus* from the NW Mediterranean Sea through body condition indices, multi-biomarker responses and histological assessment of target organs in relation to biological (ingestion and tissue accumulation) and environmental levels of pollutants, either emergent (plastics) or long-recognised pollutants (heavy metals). Both plastics and metals were consistently present in all sampled locations in both biotic and environmental samples and showed different spatial patterns. Ingested plastics, mostly fibres, were found in over 85% of the individuals, with higher values (abundance and load), as well as a higher proportion of aggregations of tangled fibres, in individuals from Barcelona. Analysis of environmental samples revealed similarities in the patterns of abundance and size distribution between ingested fibres and fibres from water, though not in the polymer composition. Overall, plastic ingestion was not significantly correlated to changes in condition indices. However, two significant correlations were observed with enzymatic responses: a positive correlation with glutathione-S-transferase activity and a negative correlation with catalase activity, suggesting an increased oxidative response in those individuals with a higher load of ingested plastics. Levels of trace metals in musculature greatly varied among locations depending on the metal species, whilst in sediment samples, higher concentrations were primarily seen in the vicinity of Barcelona. A significant relationship with the relative body condition was not observed either for heavy metals, although the concentration of organic As reported points out the need for continuous monitoring programmes for both human consumption safety issues. Finally, and most importantly, the histopathological assessment did not reveal significant alterations or pathologic conditions affecting the species in the area, thus suggesting that it may be able to cope with current pollutant levels.

5.1 Introduction

Humans are driving numerous changes at a planetary scale with the expansion of urban settlements and thriving sociocultural economies. In this Anthropocene era, the impact of human activities on natural ecosystems is undeniable and marine environments are no exception. Historical overfishing leading to the collapse of entire coastal ecosystems (Jackson et al., 2001), the increased arrival to the environment and bioaccumulation in organisms of toxic chemical pollutants, such as persistent organic pollutants or heavy metals (Cd, Pb, Hg) (Habte et al., 2015; Johnson et al., 2013), and, most recently, the death of marine megafauna, including marine mammals and turtles, as a result of macro-litter ingestion or entanglement (Anastasopoulou and Fortibuoni, 2019; De Stephanis et al., 2013), are some examples of the tremendous and numerous threats humans can create for the marine environment.

Anthropogenic stressors are unlikely to be reduced in the near future; if anything, they might be expected to increase exponentially as we face thousands of new synthetic compounds being produced (Bernhardt et al., 2017) and thus, potentially released to the environment. Not to mention the threat that climate change and the increased temperature and acidification of oceans entail for marine wildlife. Given the importance of marine ecosystems for both ecological and economic reasons, e.g. the provision of vital ecosystem services and valuable resources (Österblom et al., 2017), there is an imperious need for a continued assessment of the health condition of key components of marine ecosystems (Feist et al., 2015).

Among anthropogenic threats, plastics as an emergent contaminant have raised great awareness during the past years. Their persistence and ubiquity make them a potential hazard for marine organisms that may easily ingest them, especially when found in the form of small particles known as microplastics (defined as particles of less than 1 to 5 mm, Frias and Nash, 2019; Hartmann et al., 2019) (Andrady, 2011; Wright et al., 2013). The concern is greater for those organisms whose biological and ecological traits might lead them to increased plastic ingestion: predators that might mistake plastics for prey (e.g. marine turtles or fish) (Carson, 2013; Mrosovsky et al., 2009; Ory et al., 2017), filter-feeding organisms (e.g. mussels) (Avio et al., 2017)), or organisms with complex digestive systems that retain plastics for more extended periods (e.g. crustaceans) (Carreras-Colom et al., 2020; Welden and Cowie, 2016a).

Populations of Norway lobster (*Nephrops norvegicus*) from the Clyde Sea have been observed with remarkable concentrations of plastics in their stomach probably as a result of its accidental ingestion during their foraging behaviour (Murray and Cowie, 2011; Welden and Cowie, 2016a). These levels of ingested plastics are comparatively higher than those commonly reported for fish species in the area (Hermsen et al., 2017; McGoran et al., 2018), and pose a greater threat for the Norway lobster. Moreover, long-term exposures to plastic ingestion under controlled conditions have been observed to have a deleterious effect on their body condition (Welden and Cowie, 2016b). An observation further supported by the results of another experimental study with another decapod crustacean, the green shore crab (*C. maenas*), where a reduction in the energy budget was observed after chronic exposure to polystyrene fibres (Watts et al., 2015).

In addition to plastic ingestion, *N. norvegicus* might be exposed to other multiple stressors in the environment, of which metals are of particular relevance for environmental and human safety reasons. Contrary to plastic ingestion, strong evidence exists for the toxic impact of certain metals for which no biological role is known on crustaceans (Ahearn et al., 2004; Rodríguez et al., 2007). Moreover, increased levels of arsenic (As), cadmium (Cd) and lead (Pb) in food are considered a food safety risk and there exist national regulations on the matter.

The NW Mediterranean Sea is an area under high anthropogenic pressure with a densely populated coastline and intense industrial and agricultural land uses, coupled to heavy maritime traffic and industrial fisheries exploiting both demersal and pelagic resources. In our particular area of study, the Balearic Sea, the role of rivers in the input of terrestrial pollutants, including plastics and heavy metals, has already been highlighted (de Haan et al., 2019; Palanques et al., 2008; Sanchez-Cabeza et al., 1999; Sanchez-Vidal et al., 2018; Simon-Sánchez et al., 2019).

The comprehensive knowledge of the biology and ecology of *N. norvegicus* (see Johnson and Johnson (2013) and references therein), as well as its environmental relevance, as one of the most abundant decapod crustaceans on its depth range, and high economic value, poses an excellent opportunity for the study of the species. This study aims to provide a multidisciplinary assessment of the health status of *Nephrops norvegicus* in the Balearic Sea in relation to environmental contaminants considered relevant for their emergence (plastics) and food safety issues (heavy metals). For that purpose, an assessment of body condition indices, enzymatic activities and histology of target organs is performed, and its correlation with the presence of ingested plastics and tissue levels of heavy metals tested. Environmental levels of both contaminants are also provided for a complete risk assessment.

5.2 Materials and methods

5.2.1 Study area and sample collection

Biological and environmental samples were collected from commercial fishing vessels in June and July 2019 from three locations selected along the continental shelf break of the Catalan Coast (Fig. 1). At each location, 40 individuals of similar sizes were selected and processed in different ways. For a subsample of ten of these individuals from each location, a portion of the hepatopancreas and a portion of the tail's muscle were immediately frozen and stored at -80°C until biochemical analysis. The rest of the specimen and another ten individuals were injected and submerged in Davidson's fixative for 72h and then transferred to ethanol for stomach content analysis and histological assessment. Finally, the remaining ten individuals were immediately frozen and stored at -20°C for trace metal content analysis.

Environmental parameters (temperature in ˚C, salinity in ppt, dissolved oxygen in mg/l and turbidity in FTU) were also collected with a CTD profiler (ASTD152-ALC) at 5 m above the seafloor. Sediment samples were collected during fishing trawls using a sediment collector attached to the fishing gear, and a subsample of the sediment was kept frozen at -20°C until the analysis of granulometry, organic content and contaminants (plastics and heavy metals). Finally, net tows of ~30 min were performed to analyse the potential presence of plastics in the near-bottom water layer. Samples were collected using a WP2-net (200 µm mesh) equipped with a flowmeter that was towed horizontally at a velocity of about 2-3 knots and 5-10 m above the seafloor.

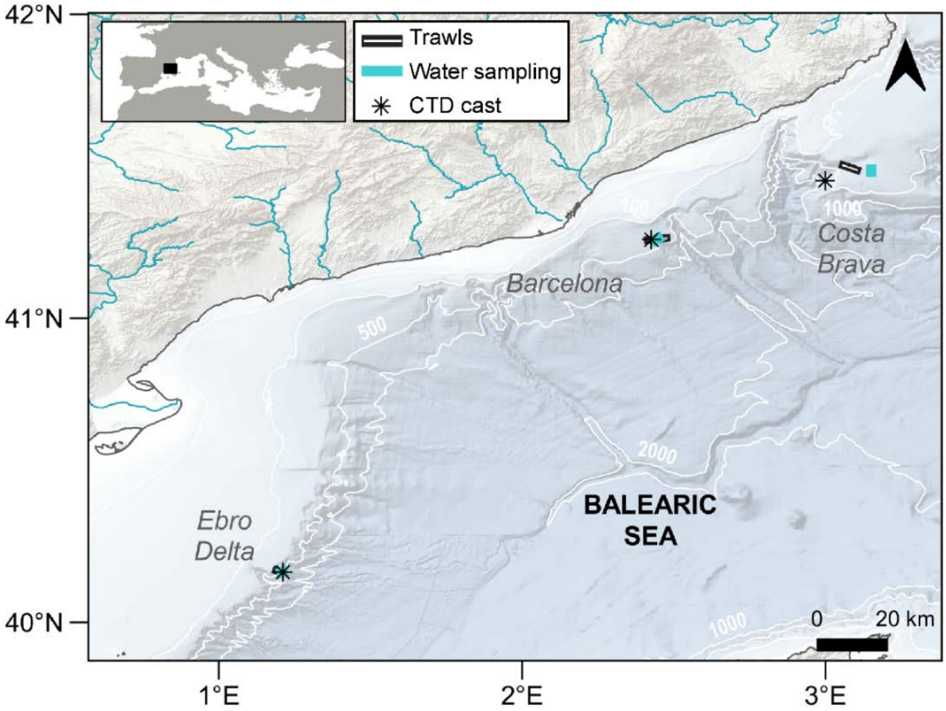


Fig 1. Sampling locations for *Nephrops norvegicus* and sediment samples (trawls), water samplings using WP-2 nets and deployment of CTD for physicochemical parameters measurement. The background map is from EMODnet Bathymetry Consortium (2020).

5.2.2 General health assessment and histological assessment

Once in the laboratory, cephalothorax length (CL, in mm), total weight without chelipeds (TW, in g) and sex were recorded, and individuals were dissected. The hepatopancreas (HW) and gonads (GW) were also weighted (in g). After screening for plastics (see section 4.2.4), stomach content was weighed (SCW, to the nearest 0.001 g). Body condition indices were calculated as follows: relative condition factor (*Kn = W/EW*, where EW is the expected weight from the weight-length regression adjusted with all individuals sampled in the study), hepatosomatic index (*HSI = HW/TW x 100*) and gonadosomatic index (only in females, *GSI = GW/TW x 100*).

A portion of targeted organs, the hepatopancreas and the gonad, the gills from one side and the first abdominal segment, were processed through routine histological techniques. Qualitative histological examination through light microscopy was conducted on haematoxylin-eosin-stained sections by comparing the tissue organisation and integrity to that reported as normal in decapod crustacean species (Bell and Lightner, 1988; Shields and Boyd, 2014). The gonadal development stage for females was also determined according to Becker et al. (2018).

5.2.3 Biochemical determinations

A portion of muscle (0.3 g) and a portion of hepatopancreas (0.2 g) were homogenised in 1 mM and 100 mM buffer phosphate, respectively, in a 1:5 (w:v) ratio using a Polytron® blender. The latter buffer containing 150 mM KCl, 100 mM EDTA, 100 mM DTT, 10 mM phenanthroline and 10 $mg \cdot ml^{-1}$ trypsin inhibitor. The homogenates were centrifuged at 10000 G for 20 min, and the supernatant used for biochemical determinations.

Assays were performed following the procedures described in past studies in the area (Antó et al., 2009; Koenig et al., 2013; Solé and Sanchez-Hernandez, 2018). All assays were carried out in triplicate at 25˚C using either undiluted or diluted supernatant, depending on the assay, and measured on a Tecan™ Infinite M200 spectrophotometer.

The activity of carboxylesterases (CbE) was measured by monitoring the hydrolysis rate of the commercial colourimetric substrate *p*-nitrophenyl acetate (pNPA) using a spectrophotometric continuous enzyme assay adapted to microplate format (Satoh and Hosokawa, 2006). The kinetic assay was performed in a medium of 50mM phosphate buffer, the substrate (1mM) and the sample. Catalase (CAT) activity was measured as a decrease in absorbance at λ = 240 nm ($\varepsilon = 40\ M^{-1} \cdot cm^{-1}$) for 1 min using H_2O_2 30% as substrate (Aebi, 1974). Glutathione-S-transferase (GST) activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate in a reaction mixture containing 1 mM CDNB and 1 mM reduced glutathione (GSH) as described in the protocol of Habig et al. (1974). The activity rate was measured as the change in OD/min for 3 min at λ = 340nm ($\varepsilon = 9600\ M^{-1} \cdot cm^{-1}$). Pentoxyresorufin-O-deethylase (PROD) activity was measured using a method adapted from Burke and Mayer (1974) as the increase in fluorescence at λ = 537 nm excitation and λ = 583nm emission over 10 min. Substrates used include 7-pentoxyresorufin and NADPH as a cofactor. A standard curve of 7 resorufin sodium salt concentrations (0-160 nM) was used to quantify activities. Acetylcholinesterase (AChE) activity was determined according to Ellman et al. (1961) at λ = 405 nm using ATC 1mM as substrate. Lactate dehydrogenase

(LDH) activity was measured following adaptation of the Vassault (1983) method using NADH (200 µM) and pyruvate (1 mM) as final well concentrations. Reading was done at λ = 340nm for 5 min. Citrate synthase (CS) activity was measured according to Childress and Somero (1990) protocol using DTNB (0.1 mM), acetyl CoA (0.1 mM) and oxalacetate (0.5 mM) at λ = 405 nm. CE, CAT, GST and PROD were analysed in hepatopancreas and AChE, LDH and CS were analysed in tail's muscle S10 fractions.

All enzymatic activities are expressed in relation to the total protein content of the sample, which was determined by the Bradford (1976) assay adapted to a microplate, using the Bio-Rad Protein Assay reagent and bovine serum albumin (BSA, 0.1-1 mg · ml^{-1}) as standard read at λ = 595nm.

5.2.4 Analysis of plastic contamination

Stomach (including the cardiac and pyloric chambers) and intestine contents were directly visually screened for the presence of plastics and other potential anthropogenic particles of similar characteristics (size and shape), i.e. cellulosic fibres or rayon (Kanhai et al., 2017; Salvador Cesa et al., 2017).

For water samples, 100 ml of the homogenised mixture was poured on a 1 mm stainless sieve and divided into two fractions. Because of the high organic matter content, the <1 mm fraction was further split into ten aliquots with a McLane rotary splitter (splitting error <4%). Subsequently, three of these aliquots were put together and went through weak alkaline digestion with 20 ml of NaOH (1 M) at room temperature for 24 h (Cole et al., 2014) to avoid the degradation of cellulosic fibres. Subsamples were split again, and two sub-aliquots were vacuum-filtered onto polycarbonate membranes (Millipore, Ø47 mm, 0.45 µm). The filters were dried overnight at 40 °C and visually inspected for the presence of potentially anthropogenic items. For the >1 mm fraction, samples underwent a direct visual inspection.

Sediment samples went through a Fenton reaction to reduce the amount of organic matter (Liu et al., 2019; Simon et al., 2018). Briefly, 50 mL of wet marine sediment were placed in beakers with 200 mL of MilliQ on a heating plate at 50°C and were continuously stirred. Samples were then oxidized by adding 36.5 mL of peroxide (50%), 15.75 mL of $FeSO_4$

(0.1 M) and 16.25 mL of NaOH (0.1 M) (Liu et al., 2019; Masura et al., 2015). The supernatant was extracted and left to settle in a glass funnel, and the remaining sediment underwent two successive density separations using saturated NaCl solution (Thompson et al., 2004). The collected supernatants were placed on a glass funnel, and after 24 h, the solids were drained. Finally, the three liquid phases collected for each sample (one from the Fenton's reaction and two after density separation procedures) were merged and vacuum-filtered onto polycarbonate membranes (Millipore, Ø47mm, 0.45μm). The filters were dried overnight at 40°C and visually inspected for the presence of potentially anthropogenic items.

Measures were taken to prevent and reduce potential contamination. Dissection of specimens was performed in a safety cabinet, all material used was rinsed with filtered water (50 μm) and checked before use, and screening of digestive contents was performed using a stereomicroscope fully covered by an isolation device. Air controls (Petri dish filled with water) placed close to the sample being screened inside the isolation device were used to monitor airborne contamination. Water and sediment sample processing was performed in a clean laboratory (positive pressure), all material used was previously rinsed and distinctive orange lab coats used at all times. Procedural blanks were performed to check for potential contamination.

All particles suspected of anthropogenic origin, including plastics, were separated, counted, observed with optical microscopy for further characterization and measured using a MicroComp Integrated Image Analysis System. The polymer composition was further identified as described in Carreras-Colom et al. (2020) through ATR-FTIR or when their small size rendered this impossible, by means of μFTIR at the Scientific and Technological Centres (CCitUB, University of Barcelona).

Anthropogenic items were classified into synthetic (plastic) and cellulosic (potentially man-made) items, and their concentration levels were calculated in terms of abundance (number of particles) and load (as the sum of lengths of fibres, in mm, and the sum of areas of fragments and films, in mm^2) per individual or unit of water and sediment sampled, i.e., m^3 and ml, respectively).

5.2.5 Determination of heavy metal content

Muscle portions (no cuticle) were oven-dried until constant weight and homogenised. Subsamples of 0.2 g were acid-digested (5 ml HNO_3 and 0.5 ml HF) in an automated microwave system (Milestone Ultraware) using closed Teflon vessels at 240 ˚C for 15 minutes. Similarly, sediments were oven-dried, and then subsamples of 0.2 g were acid-digested following the EPA3051a method with 5 ml HNO_3 and 2 ml HCl at 200 ˚C for 15 minutes.

Following acid digestion, concentrations of Cd, Zn, Li and Ti (only muscle), and Pb, Cu, Co, Cr, Ni, Mn, Al, As and Fe (both in muscle and sediment) were analysed by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500CE ICP-MS; Agilent Technologies, Los Palos, CA). Certified reference materials (ERM®-BB422 Fish Muscle, Joint Research Centre; BCR-701 Lake Sediment, European Commission) and laboratory blanks were included in each batch. Percentage recovery from certified reference materials was rendered acceptable (ranging between 85-115%), and no corrections were applied on concentration levels from problem samples. Values are given in dry weight unless stated otherwise.

5.2.6 Data analysis

All statistical analyses were performed in R version 3.6.3. Differences among locations for contaminants in biological samples (prevalence, abundance and load of anthropogenic items and for tissue levels of metals) and health parameters (body condition indices and biomarkers response) were tested using GLM (logistic and Poisson regressions depending on the variable) with CL as a covariable. Differences in the polymer composition and size distribution of ingested plastics among locations were tested through a permutational analysis of variance (PERMANOVA; "vegan" package) and an adaptation of the Kolmogorov-Smirnoff test, respectively. Similarly, differences among locations for the concentration of trace metals were tested by GLM to account for CL as a covariable. GLM models were also used to test the correlation between health parameters (condition indices and enzymatic activities) and contaminants (plastics and trace metals) while accounting the individual's size and site differences. Potential patterns were then visualized through Principal

Component Analysis (PCA) and their relation with environmental conditions (physicochemical parameters and environmental levels of contaminants) further explored with Redundancy Analysis (RDA). A backwards stepwise selection procedure based on a permutational test was used to select the most explanatory variables. For all statistical tests, significance level was set at $p < 0.05$, though marginal differences ($0.1 > p > 0.05$) are also discussed.

5.3 Results

A total of 90 adult individuals in the inter-moult stage were included in this multidisciplinary study. Of these 60 were included in the analysis of the presence of plastics and other anthropogenic items, and another 30 were analysed for the presence of trace metal concentrations.

5.3.1 Levels of plastics and other potentially anthropogenic items

Overall, 83.3% of the individuals were observed with at least one synthetic fibre in their stomach and 11.7% with cellulosic fibres that might have an anthropogenic origin. In some individuals (20% in the Costa Brava and Barcelona sampling locations), fibres were found tangled up in balls occupying a small percentage volume of the stomach (c.a. < 10 %) (Table 1). Only one individual was observed with a black film-like particle made of polyethylene in its stomach, and from all intestine contents analysed, only one fibre made of cellulosic material and with traces of black original colour was identified.

Differences among sampling locations were not observed for the values of prevalence of either synthetic, cellulosic or tangled fibres ($p > 0.05$). None of the individuals from the Ebro Delta had a ball of fibres in their stomach. Significant differences regarding the mean abundance and load of synthetic fibres were observed among locations (GLM; $p < 0.01$), with Barcelona showing higher values compared to the other locations (abundance: $z = -6.424$ and -7.7, $p < 0.01$; load: $z= -5.78$ and -7.82, $p < 0.01$). Moreover, tangled fibres in Barcelona were also more abundant and made up for a higher total load compared to the

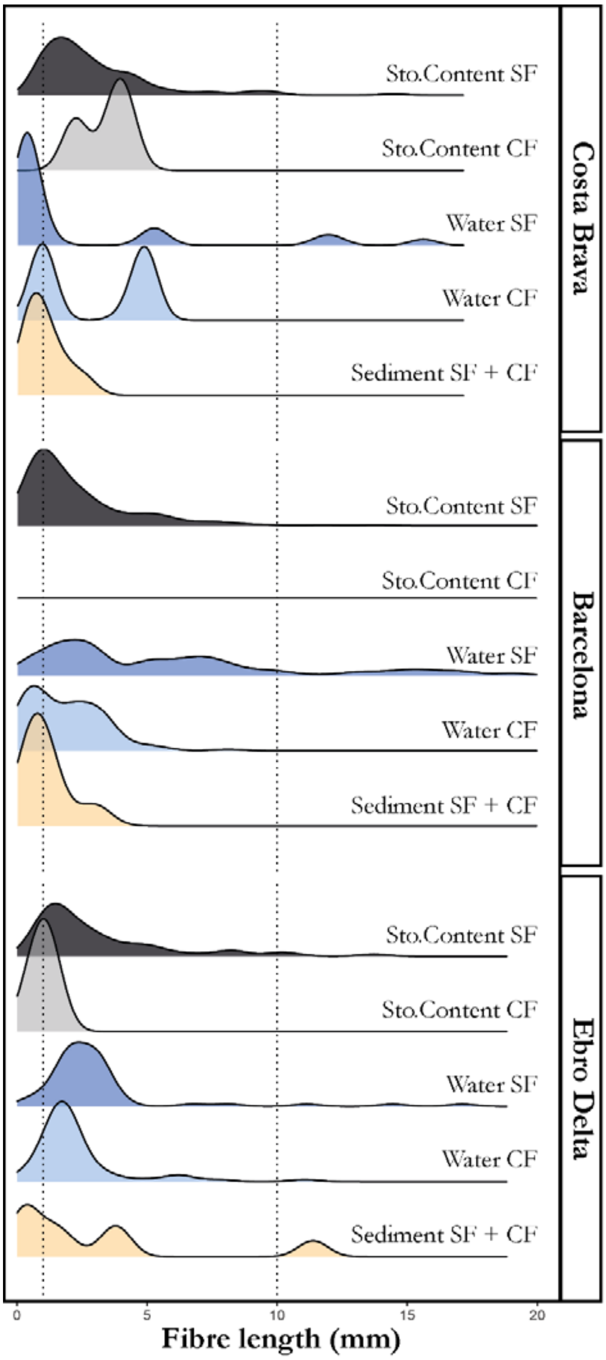
Costa Brava (abundance: z = -7.12, p < 0.01; load: z = -7.70, p < 0.01). Mean values of all parameters related to ingestion of anthropogenic items can be found in Table 1.

Table 1. Prevalence of plastic items in digestive contents of *N. norvegicus* and environmental samples (water and sediment) according to location. *n* = number of individuals/samples analysed. Different superscript letters among rows indicate differences among locations.

		Girona	Barcelona	Delta
Stomach contents	*n*	*20*	*20*	*20*
Synthetic fibres	%	75 ^{a}	85 ^{a}	90 ^{a}
	$(n \cdot ind^{-1})$	6.20 ± 6.80 ^{a}	12.55 ± 20.78 ^{b}	5.20 ± 4.44 ^{a}
	$(mm \cdot ind^{-1})$	19.46 ± 21.90 ^{a}	28.45 ± 41.88 ^{b}	16.73 ± 14.67 ^{a}
Cellulosic fibres	%	15 ^{a}	5 ^{a}	15 ^{a}
	$(n \cdot ind^{-1})$	0.15 ± 0.37 ^{a}	0.05 ± 0.22 ^{a}	0.15 ± 0.37 ^{a}
	$(mm \cdot ind^{-1})$	0.51 ± 1.29 ^{a}	0.02 ± 0.1 ^{a}	0.15 ± 0.4 ^{a}
Tangled fibres	%	20 ^{a}	20 ^{a}	0
	$(n \cdot ind^{-1})$	1.55 ± 4.05 ^{a}	6.45 ± 17.96 ^{b}	0
	$(mm \cdot ind^{-1})$	6.20 ± 13.73 ^{a}	14.20 ± 37.32 ^{b}	0
Fragments and films	%	0	5	0
	$(n \cdot ind^{-1})$	0	0.05 ± 0.22	0
	$(mm \cdot ind^{-1})$	0	0.07 ± 0.33	0
Near-bottom water layer	*n*	*1*	*2*	*2*
Synthetic fibres	$(n \cdot m^{-3})$	0.12	1.25 ± 0.72	0.09 ± 0.08
	$(mm \cdot m^{-3})$	1.28	10.14 ± 4.52	0.43 ± 0.17
Cellulosic fibres	$(n \cdot m^{-3})$	0.13	0.57 ± 0.44	0.10 ± 0.03
	$(mm \cdot m^{-3})$	0.38	0.85 ± 0.09	0.4 ± 0.23
Fragments and films	$(n \cdot m^{-3})$	0.13	0.10 ± 0.13	0.014 ± 0.005
	$(mm \cdot m^{-3})$	0.0004	0.007 ± 0.008	0.002 ± 0.002
Sediment	*n*	*1*	*1*	*1*
Synthetic fibres	$(n \cdot ml^{-1})$	0.04	0.12	0.02
	$(mm \cdot ml^{-1})$	0.04	0.16	0.13
Cellulosic fibres	$(n \cdot ml^{-1})$	0.14	0.02	0.07
	$(mm \cdot ml^{-1})$	0.15	0.03	0.10
Fragments and films	$(n \cdot ml^{-1})$	0	0	0.06
	$(mm \cdot ml^{-1})$	0	0	0.003

In terms of fibre characterization, significant differences were identified among locations for the fibre size distribution of ingested synthetic fibres (K-S: D = 0.21, p < 0.001 with individuals from Barcelona showing a higher contribution of small fibres (<1mm) compared to those from the Costa Brava and the Ebro Delta with a higher contribution of fibres > 1 mm

(Fig. 2). Mean fibre length was also smaller in Barcelona (2.26 ± 2.11 mm) compared to other samplings (3.14 ± 2.78 in the Costa Brava, and 3.19 ± 2.83 mm in the Ebro Delta, respectively; K-W: $X^2 = 30.96$, $p < 0.001$). No significant differences were observed in the relative composition of polymers of fibres ingested among locations (PERMANOVA; pseudo-F = 1.7744, p = 0.11).



◂ **Fig. 2.** Size distribution of synthetic (SF) and cellulosic fibres (CF) found in stomach contents of *N. norvegicus* and water and sediment samples according to the sampling location. Plot elaborated with R packages 'ggplot2' and 'ggridges' using the Kernel density distribution to highlight the potential patterns and avoid the confounding effect of different concentration measures used for each compartment (stomach contents and water and sediment samples). Given the small number of fibres characterized in sediment samples, SF and CF were grouped.

Among environmental samples, highest loads of synthetic fibres were observed in Barcelona for water and in Barcelona and the Ebro Delta for sediment samples (Table 2, Fig. 3). Cellulosic fibres were equally found in water samples from the three locations but were more abundant in the Costa Brava and Ebro Delta sediments than in Barcelona. Contrary to ingested items, fragments and films were identified in water samples of all three locations and in sediments from the Ebro Delta. These items of irregular shape were mainly made of PE and PET, and none of them surpassed the 5 mm of diameter (longest diagonal measured). Regarding fibres, the polymer composition was slightly different to that of ingested fibres and varied considerably compared to ingested fibres and even between water and sediment samples from the same location. Either PA or cellulose were the dominant polymers in water samples, whilst in sediment samples, it was mainly cellulose, and PET or PP (Table 2). Finally, in terms of size distribution, two main patterns were observed: (1) synthetic fibres had a higher contribution of longer fibres, especially in water samples, and (2) density curves of ingested fibres seemed more similar to those of fibres identified in water samples rather than sediment samples (Fig. 2).

Table.2. Polymer composition (in percentage over the total load) of fibres found in stomach contents and water and sediment samples. For stomach contents, mean values (± standard deviation) calculated from the proportions observed in each individual are given.

	PA	PE	PET	PP	Acr	Cel
Stomach contents						
Costa Brava	27.8 ± 34	0 ± 2.5	51 ± 39.6	3.5 ± 7.3	12.3 ± 14.3	5.5 ± 0.2
Barcelona	29.4 ± 14.6	0.6 ± 0	46.8 ± 36.8	1.8 ± 17.8	21.4 ± 29.1	0.1 ± 3.8
Ebro Delta	9.1 ± 34.6	0 ± 0	44.8 ± 31.4	6.2 ± 7.8	38.6 ± 36.8	1.3 ± 12.9
Water samples						
Costa Brava	70.7	0.0	1.2	1.3	3.0	23.9
Barcelona	84.9	0.0	0.7	2.2	4.4	7.7
Ebro Delta	23.3	3.5	11.9	0.2	13.0	48.1
Sediment samples						
Costa Brava	0.0	0.0	0.0	0.0	18.7	81.3
Barcelona	0.0	16.7	52.7	0.0	14.0	16.7
Ebro Delta	5.5	0.0	0.0	49.5	0.0	45.1

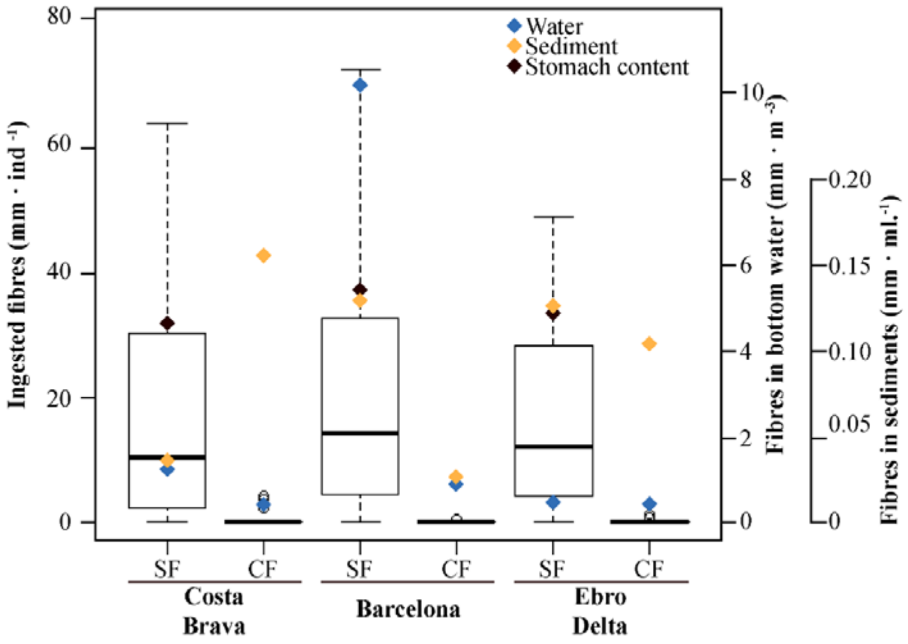


Fig. 3. Values of fibre load (synthetic, SF, and cellulosic, CF) in stomach contents of *N. norvegicus* (white boxplots, in mm · ind^{-1}) and water (blue, in mm · m^{-3}) and sediment (orange, in mm · ml^{-1}) samples. Diamonds indicate mean values.

5.3.2 Levels of trace metal concentrations

Levels of trace metal concentrations in muscle and sediment samples are given in Table 3. Significant differences among locations in the levels of trace metals in abdominal muscle were observed with higher levels of Li, As, and Cd in individuals from the Costa Brava, whereas higher values of Al, Ti, Mn, Fe, Co, Cu and Zn were observed in the Ebro Delta area (ANOVA; $F_{2,26} = 3.77 - 11.93$, $p < 0.05$). Values of Cr might be higher in the Ebro Delta since it was the only area where it was observed above the detection limit (0.31 ppm) (no statistical tests could be performed). No significant differences were observed for Ni or Pb among locations, and no significant relationships with size or sex were found for the concentration of any metal analysed when considering individuals from each sampling location alone ($p > 0.05$). The resulting RDA performed on the data matrix of trace metal data in *N. norvegicus* with stepwise selection of physicochemical parameters of the water and sediment suggests that the percentage of organic matter and silt and clay rather than other physicochemical properties, are the most explanatory variables for the trends in heavy metals observed

Table 3. Trace metal concentrations in tail muscle of *Nephrops norvegicus* and sediments from the three locations sampled. Values are given in mg · kg d.w.$^{-1}$. Different superscript letters indicate differences among locations in the levels of trace metals in *N. norvegicus*.

	Li	Al	Ti	Cr	Mn	Fe	Co
N. norvegicus							
Costa Brava	0.61 ± 0.15 [a]	47.0 ± 52.7 [a]	3.45 ± 2.92 [a]	BDL	3.84 ± 0.85 [a]	32.7 ± 27.2 [a]	0.06 ± 0.01 [a]
Barcelona	0.41 ± 0.2 [b]	64.2 ± 46 [b]	3.74 ± 1.53 [ab]	BDL	5.21 ± 1.8 [ab]	31.1 ± 12.6 [a]	0.06 ± 0.01 [a]
Ebro Delta	0.51 ± 0.08 [ab]	77.6 ± 16.2 [b]	5.09 ± 1.07 [b]	0.44 ± 0.2	5.41 ± 1.44 [b]	60.4 ± 21.3 [b]	0.08 ± 0.02 [b]
Sediment							
Costa Brava	-	26732 ± 2588	-	40.2 ± 2.6	509.98 ± 23.42	28199 ± 2198	9.81 ± 0.24
Barcelona	-	24721 ± 967	-	41.6 ± 0.6	920.94 ± 315.64	32874 ± 1538	11.57 ± 0.26
Ebro Delta	-	11414 ± 6606	-	22.5 ± 9.4	491.63 ± 235.79	27315 ± 2289	9.06 ± 1.34

	Ni	Cu	Zn	As	Cd	Pb
N. norvegicus						
Costa Brava	0.22 ± 0.09 [a]	16.2 ± 5.2 [a]	53.02 ± 3.20 [a]	109.69 ± 21.94 [a]	0.023 ± 0.006 [a]	0.28 ± 0.23 [a]
Barcelona	0.17 ± 0.07 [a]	19.6 ± 7.4 [ab]	56.59 ± 2.56 [b]	112.64 ± 13.88 [a]	0.020 ± 0.004 [b]	0.32 ± 0.17 [a]
Ebro Delta	0.20 ± 0.05 [a]	23.5 ± 7.8 [b]	57.55 ± 2.31 [b]	76.74 ± 18.08 [b]	0.015 ± 0.004 [b]	0.28 ± 0.13 [a]
Sediment						
Costa Brava	37.19 ± 0.26	13.72 ± 0.41	-	14.82 ± 2.22	-	25.76 ± 1.43
Barcelona	38.55 ± 0.48	18.15 ± 0.30	-	25.05 ± 0.42	-	41.99 ± 0.49
Ebro Delta	24.87 ± 4.30	9.31 ± 6.37	-	26.76 ± 1.20	-	28.90 ± 8.48
ERL[a]	20.9	34	150	8.2	1.2	46.7
ERM[a]	51.6	270	410	70	9.6	218
TET [b]	61	86	540	17	3	170

[a] Long et al. (1995)

[b] MacDonald et al. (2000)

among locations (Fig. 4). Sediments from Barcelona showed a higher percentage of organic matter and silt and clay (Table 4). Trends in the levels of trace metals among locations were only partially shared by trends in the concentrations of the same elements in the sediment. Barcelona showed the highest concentrations for all metals, with equally high levels in Al, Cr, Ni and Cu in the Costa Brava, and As in the Ebro Delta (Table 3).

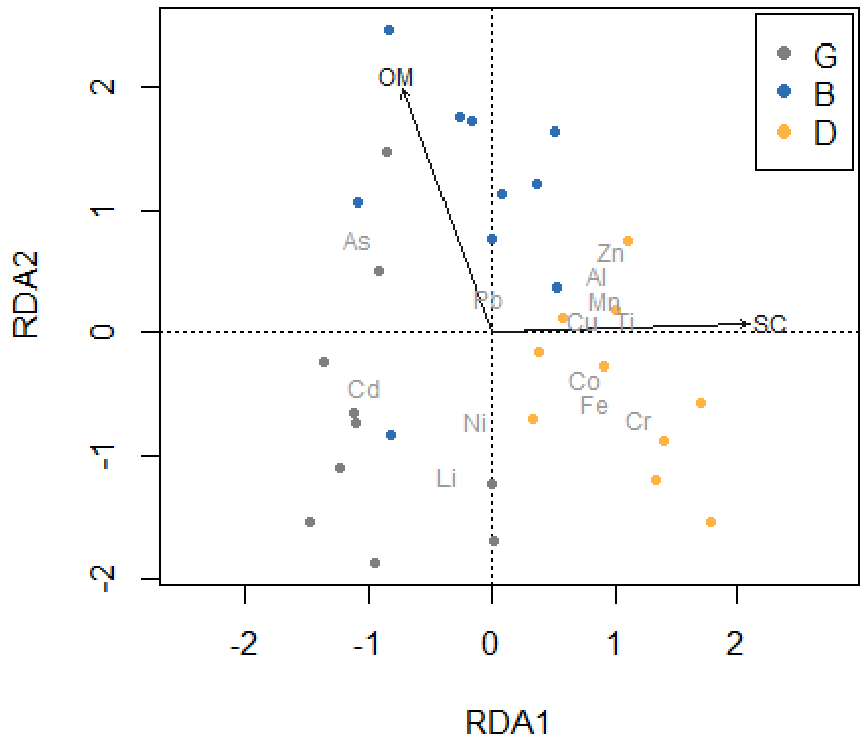


Fig. 4. Redundancy analysis on tissue levels of heavy metals in *N. norvegicus* constrained by the physicochemical parameters of water and sediment, from which were selected the percentage of organic matter (OM) and the percentage of silt and clay (SC) as the most significant for the ordination. Colours refer to sampling locations of individuals (G: Costa Brava; B: Barcelona; D: Ebro Delta).

Table 4. Environmental conditions of the three sampled locations (CB: Costa Brava, BC: Barcelona; and ED: Ebro Delta) including physicochemical parameters of bottom water (mean values for ca. 5m above the seafloor) and sediment. T: temperature; S: salinity; DO: dissolved oxygen; Tu: turbidity; OM: organic matter; SC: silt and clay; SA: sand.

Sampling location	Depth (m)	T (˚C)	S (ppt)	DO (mg/L)	Turbidity (FTU)	$CaCO_3$ (%)	OM (%)	SC (%)	SA (%)
CB	404	13.76	38.63	6.52	0.43	31.31	13.90	34.17	65.83
BC	438	13.66	38.61	6.58	0.62	20.34	15.90	40.87	59.13
ED	339	13.73	38.60	6.58	0.15	23.73	13.36	50.61	49.39

5.3.3 Histological assessment

Histopathological assessment of target organs (gills, hepatopancreas and abdominal muscle) did not reveal relevant histopathological alterations except for a granuloma-like structure in gills (Fig. 5A), found in one individual from Barcelona, and an haemocytic nodule within an extensive haemocyte infiltration in muscle tissue (Fig. 5B) in one individual from the Ebro Delta. No apparent evidence of the aetiological agents was found in any case. Although the intestine was not targeted in the histopathological assessment, small portions were observed in preparations of muscle or hepatopancreas. Incidentally, aggregations of elongated to ovoid yeast-like cells within cyst-like spaces were observed in the intestinal submucosa of at least five individuals, one from Barcelona and four from the Ebro Delta (Fig. 5C). A layer of fibrous connective tissue was observed surrounding these structures, suggesting a potential encapsulation host response. Since the intestine could not be evaluated for all individuals, the prevalence of this finding could not be appropriately estimated.

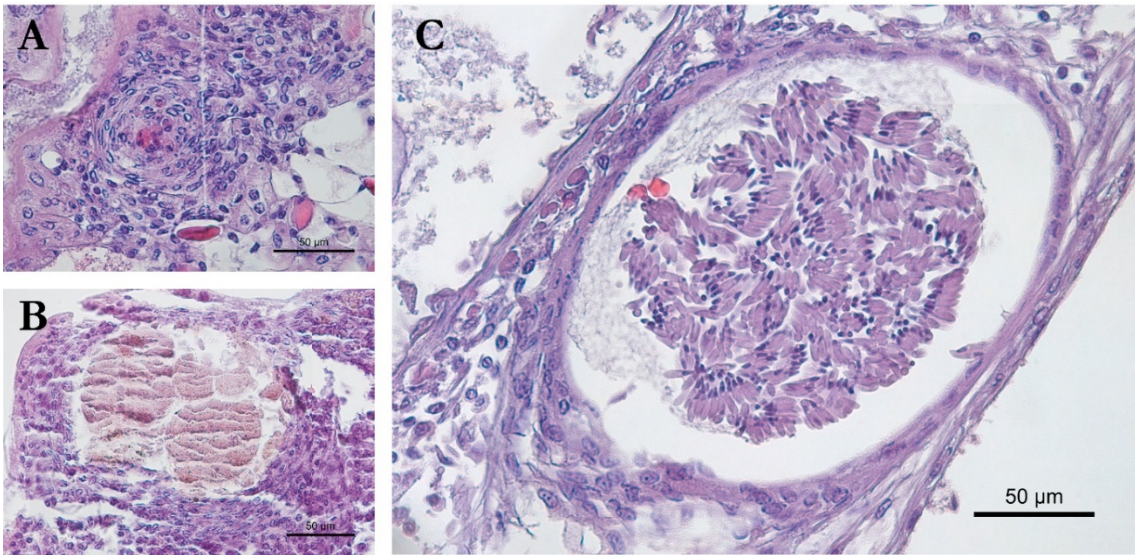


Fig. 5. Histological findings observed in *N. norvegicus*: (A) granuloma-like structure found in gills. B) structure with remains of degenerated material observed in gills, (B) remains of an haemocytic nodule within an extensive haemocyte infiltration in muscle tissue, (C) elongated to ovoid yeast-like cells fond inside cyst-like structures surrounded by a layer of host cells in the intestinal submucosa. Scale bars provided for reference of size. Standard H&E stain used.

On the whole, the gill epithelium showed a regular appearance and no alterations of cell morphology were identified in hepatopancreas or muscular cells either. The low prevalence of the findings described limited further analysis of any potential relationship with size, other health biomarkers (condition indices and enzymatic activities) or contaminant levels.

5.3.4 Condition indices

Significant differences, unrelated to the size of the individual, were observed for HSI and GSI among sampling locations. Individuals from the Ebro Delta showed the highest values of HSI ($F_{2,56}$ = 9.3808, $p < 0.001$) and Barcelona females showed the highest GSI values ($F_{2,27}$=4.54, $p = 0.02$). All females, regardless of their sampling location, were considered to be in a late ovary maturation stage with enlarged dark green ovaries that extended to the first abdomen segments (macroscopic observations during dissection) and with vitellogenic oocytes showing densely packed yolk vesicles (microscopic observation; stage 4 according to Becker et al. (2018) (Table 5). Stomach fullness was also significantly higher in Barcelona compared to the Costa Brava individuals ($F_{2,57}$ = 5.9035, $p = 0.005$). No differences were observed for the relative condition factor ($p > 0.05$), or between condition indices or stomach fullness and the abundance and load of ingested plastics ($rho < 0.2$, $p > 0.05$).

Table 5. Summary of the biological parameters (mean and standard deviation) of individuals of *Nephrops norvegicus.* CL = cephalothorax length; n = number of individuals. F = female. Stages of maturity defined from I to V (Rotllant et al., 2005); Kn = relative condition factor; HSI = hepatosomatic index; GSI = gonadosomatic index.

	Costa Brava	Barcelona	Ebro Delta
n	20 (9)	20 (9)	20 (12)
Size range (CL, mm)	25.0 – 34.7	32.5 – 40.7	28.0 – 36.2
Kn	0.97 ± 0.13 [a]	1.05 ± 0.11 [a]	1.00 ± 0.13 [a]
HSI	4.04 ± 0.72 [a]	4.29 ± 0.78 [a]	4.85 ± 0.84 [b]
GSI	4.37 ± 1.71 [a]	6.48 ± 1.94 [b]	4.52 ± 1.47 [a]
Maturity stage	IV	IV	IV
Stomach fullness	1.97 ± 0.75 [a]	1.11 ± 0.89 [b]	1.48 ± 1.02 [ab]

15.3.5 Biomarker's response

No site-related differences were observed for most biomarkers, except LDH, CbE and GST, each displaying a different pattern (Table 6). LDH activities were higher in Barcelona and the Ebro Delta individuals compared to those from the Costa Brava ($F_{2,27} = 7.35$, $p = 0.003$), CbE showed higher levels in the Costa Brava instead ($F_{2,21} = 6.91$, $p = 0.005$) and GST activities were marginally higher in Barcelona ($F_{2,21} = 3.30$, $p = 0.057$), the past two cases compared to the Ebro Delta.

Of all biomarkers analysed, three were significantly correlated with the fibre load of the individuals: GST, CAT and, partially, LDH. Synthetic fibre load was positively related to GST ($t = 2.84$, $p = 0.01$) and negatively to CAT ($t = -3.14$, $p = 0.005$). Finally, a significant interaction was observed for LDH between fibre load and individuals' size ($F_{1,16} = 4.75$, $p = 0.04$). When only bigger individuals were considered (CL > 31.6 mm), LDH was positively related to fibre load ($t = 2.18$, $p = 0.045$). PCA analysis performed on the biochemical determinations response data matrix showed a great ordination of individuals based on their enzymatic response. This ordination partially supported the correlations mentioned above with synthetic fibre load, with individuals with a higher load positioned mostly on the left and bottom left corners, being therefore partially associated negatively with CAT and positively with GST but also LDH and CS activities (positively) (Fig. 6). RDA performed afterwards, including other environmental conditions, did not reveal any significant patterns (significance levels of the ordination $p>0.05$).

5.4 Discussion

Our study characterises the levels of plastic ingestion and tissue levels of heavy metals in *N. norvegicus* from the NW Mediterranean Sea and provides a general assessment of the health condition of these individuals with particular attention to the potential correlation with the levels of contaminants analysed and their spatial trends.

5.4.1 Plastic ingestion in *N. norvegicus*

The high levels of ingested plastic fibres and common presence of tangled balls observed in the Balearic Sea fall into the range of the levels reported in several areas for this species (thoroughly reviewed in CHAPTER 6) as well as other crustacean species (Devriese et al., 2015; McGoran et al., 2020). Despite showing similar levels of abundance, it is worth noting the vast contrast with the results reported by Cau et al. (2019) in the Tyrrhenian Sea or Martinelli et al., (2021) in the Adriatic Sea in terms of shape, since in both studies a significant larger proportion of fragments and films was identified (86 and 57%, respectively). Differences in the methodological approach might be responsible for these differences to some extent (e.g., textile fibres not accounted because of their potential airborne contamination origin, identification of particles in a much smaller size range), but further research would be needed to properly understand such differences.

Spatial differences along the Catalan coast match the patterns already described at shallower areas (~100 m) in red mullet *Mullus barbatus* (Rodríguez-Romeu et al., 2020), for which higher values were described in Barcelona compared to the Costa Brava, and at deeper depths (~800 m) for the deep-sea shrimp *Aristeus antennatus*, for which high levels of plastic ingestion in the vicinity of Barcelona were repeatedly found compared to other locations along the coast, including the Costa Brava and Ebro Delta locations (Carreras-Colom et al., 2020). In our case, site-related differences in the levels of ingested plastics are further supported by the differences in the levels of environmental plastics, for which higher values were also observed near Barcelona, thus suggesting that this area, characterised by a high population density and industrialisation level, might have an increased arrival of such contaminants.

The association between the anthropization level of the surrounding land areas and the increased presence of synthetic fibres in the environment has been observed in other plastic studies (Alomar et al., 2016; Ruiz-Orejón et al., 2018). For instance, Jambeck et al. (2015) estimated that over 4.8 MT of plastic entered the ocean from land in 2010 alone. Particular emphasis has been placed on the role of rivers and storm-water runoffs on the transport of plastics into the ocean (Galafassi et al., 2019; Lebreton et al., 2017). In the Mediterranean Sea, in addition to heavily urbanised watersheds, most rivers also receive the effluents of numerous wastewater treatment plants (WWTPs), which are viewed as important pathways into the environment for synthetic fibres that originate in urban households (Galafassi et al., 2019 and references therein). The dominance of synthetic fibres made of acrylic and polyethylene terephthalate (polyester) polymers (over 75% of the ingested fibres in Barcelona), in addition to the abundant blue-dyed cellulosic fibres observed in sediments resembling characteristic fibres of Jeanswear (De Wael et al., 2011; Rodríguez-Romeu et al., 2020), provide further support to the likeliness of a textile origin for an important part of the fibres identified. However, sea-based sources such as the release of fibres from aquaculture and fishery equipment should not be ruled out either as both PA and PP, two commonly used polymers in fishing ropes and nets (Salvador Cesa et al., 2017), were also identified in variable proportions. In general terms, the polymer composition observed matches that of the global composition observed for synthetic fibres in both surface water and sediments, in order of abundance: PET > PA and acrylic fibres > PP and PE (Sanchez-Vidal et al., 2018; Suaria et al., 2020).

Local hydrodynamics conditions of the continental shelf and shelf break in the area are known to enhance the transport of organic particulate matter and other contaminants into the ocean (Sanchez-Vidal et al., 2008). Several studies have pointed out the seafloor as the ultimate fate of plastics and marine litter in broad terms (Fischer et al., 2015; Mordecai et al., 2011; Sanchez-Vidal et al., 2018). Our results on sediment concentrations are below those previously reported along the submarine canyon and adjacent continental shelf in the Costa Brava by Sanchez-Vidal et al. (2018), who reported between 0.2 and 0.7 fibres of synthetic composition per ml of sediment analysed (values calculated from the number of fibres reported · 50 ml^{-1}). In this work, they also highlighted the presence of cellulosic fibres (up to 1.4 fibres · ml^{-1}) whose abundance greatly surpassed synthetic fibres, as happens in our samples from the Costa Brava and the Ebro Delta. Very few studies targeting deep-sea sediments of similar depths exist, limiting further discussion on whether these levels of plastic pollution in sediments might or might not be typical near urbanised areas. Likewise, only one study has characterised the presence of plastics in deep waters so far (Courtene-Jones et al., 2017), and even though they sampled the remote location of the Rockall Through at depths > 2200 m, they found values well above the concentrations observed in our study (70.8 fibres · m^{-3}). Comparisons for areas of similar characteristics, close to urban areas, are limited to observations on surface waters. For instance, Lefebvre et al. (2019) reported values of 0.23 fibres · m^{-3} in the Gulf of Lions, and de Haan et al. (2019) reported highly variable concentrations of plastic particles along the Catalan Coast with average values between 0.039 and 2.057 items · m^{-3}. Values reported in both studies are comparable to those observed in bottom waters in the present work. However, one of the most recent and extensive assessments of synthetic fibres in surface waters found a mean value of 4.6 fibres · $litre^{-1}$, and even though over 90% of these fibres were rendered of cellulosic or natural composition, the resultant values for synthetic fibres still greatly surpass our estimated values in bottom waters. This poses the question of whether deep environments may exhibit increased concentrations compared to surface waters or not.

Given the size distribution and patterns in abundance and load observed among sites for environmental and ingested values of plastics, it seems like water would have a more critical role in the exposure to the ingestion of plastics in *N. norvegicus.* Until now, it had been hypothesised that the most plausible route of entry for plastics in this benthic species would be passive ingestion while preying on epibenthic and endobenthic fauna (Carreras-Colom et al., 2018). However, the similarities between ingested plastics and those found in bottom water samples suggest otherwise. Suspension feeding in adults of *N. norvegicus* was described some time ago, although it was initially thought to be a strategy adopted by egg-bearing females during the long breeding season to avoid leaving the burrow (Loo et al., 1993). Later on, Santana et al. (2020) provided evidence that suspended particulate organic matter significantly contributed to its diet, at times by almost half of the diet (47%), of both females and males from Clew Bay (Ireland). In the absence of more information on whether Mediterranean populations might share this feeding strategy and to what extent, it could be hypothesised that plastics suspended in the bottom water might be ingested during suspension-feeding, thus resulting in shared similarities between plastics from the water and those observed in stomach contents.

In general, both levels of ingested and environmental plastics correlated well and succeeded in pointing out the area of Barcelona as the area with the highest impact of plastic pollution, whilst the Costa Brava showed higher levels of cellulosic fibres, for which their potential impact is even more questionable (Mateos-Cárdenas et al., 2021; Remy et al., 2015). Simultaneous analyses of both biological and environmental field samples also prove this species' value as a bioindicator for local levels of plastic fibre pollution, as already proposed by several authors (Cau et al., 2019; Fossi et al., 2017). It is more so if it is considered a species of stationary nature with limited mobility of the adults (Bonanno and Orlando-Bonaca, 2018).

5.4.2 Heavy metals

Tissue levels of heavy metals did not display a clear spatial trend and, contrary to the trends observed in plastic ingestion, individuals from Barcelona rarely displayed the highest levels of any of the metal species analysed. Marine invertebrates accumulate trace metals mainly from two different sources, seawater and diet, and the specificity of the metal is key in determining their availability and uptake (Eriksson et al., 2013). With no further information on trace levels of other tissues (i.e., hepatopancreas) and given the patterns in sediment levels discussed afterwards, it is hard to elucidate the reasons behind the differences observed. In general terms, the levels identified fall in the range of those previously reported by Canli and Furness (1993) except Cd, here reported in much lower concentrations.

Analysis of Ti was included for future reference given the growing interest in TiO_2 in the plastic field, for it is widely used as a plastic additive (pigment), and because of food safety reasons since the European Food Safety (EFSA) does no longer consider it safe for use as a food additive (EFSA, 2021). As of Cd and Pb levels, for which there exist national regulations for food (Commission Regulation (EC) No 1881/2006), concentrations observed in abdominal muscle (0.004 and 0.26 mg · kg^{-1} in w.w., respectively, in wet weight) were below those established by the EFSA for crustaceans (0.50 mg · kg^{-1} in w.w.). The most concerning values regarding food safety issues would be those observed for As in abdominal muscle, the main edible part of langoustines, even though there are no established threshold levels. Our observed values (overall mean values of 21.33 mg · kg^{-1} in w.w.) are similar to those reported in a risk assessment study performed in Catalonia (Spain) on the presence of As in marketed products (Fontcuberta et al., 2011). They reported mean values of 16.1 mg · kg^{-1} in w.w. in red shrimp and 7.83 mg · kg^{-1} in w.w. in Norway lobster. The lower values reported in *N. norvegicus* might be related to their potential origin. Red shrimp (c.f. *Aristeus antennatus*) would come from local fisheries (i.e. the same from our study) whilst marketed *N. norvegicus* are mostly imported from the Atlantic Sea, which might be subjected to lower levels of pollution (Ferrante et al., 2019).

In terms of risk exposure for humans, it should be noted that these values refer to total As, but it is its inorganic form that is considered to be a human carcinogen. Based on the results of Fontcuberta et al. (2011), in these species' inorganic As may represent only 2-3% of total As. Moreover, when dietary intake of other sources of inorganic As, as well as their bioaccessibility, were taken into account, they estiamted that rice, rather than shellfish, would be the main contributor for inorganic As in the population of the study area.

Levels of trace metals in sediments were elevated in the Barcelona area, evidencing the impact of these intense industrial and urban land uses again. Similar studies assessing the presence of heavy metals along the Catalan coast have pointed out the same area for their higher concentration values (Pinedo et al., 2014), and others have highlighted the role of riverine inputs (Canals et al., 2006; Palanques et al., 2020, 2017). Even though stricter environmental regulations and the increase in WWTPs and other corrective measures applied in the last decades have contributed to a decline in trace metal pollution, monitoring programs have shown that coastal water quality can still be affected by rain events (Palanques et al., 2017).

Overall, levels observed for most metals were similar to or below those previously reported levels in the area (Jordana et al., 2015; Palanques et al., 2017; Pinedo et al., 2014). When comparing with probable effect concentrations established as reference levels to predict toxicity levels (i.e., effects range-low (ERL), effects range-median (ERM) and toxic effect threshold (TET)) (Long et al., 1995; MacDonald et al., 2000), only Ni and As were above the ERL levels. Moreover, concentrations of As in sediments from Barcelona and the Ebro Delta surpassed the established TET levels, thus suggesting that sediments in the area are heavily polluted and adverse effects on sediment-dwelling organisms might be expected (MacDonald et al., 2000). However, current research is being undertaken to re-evaluate the ecological thresholds in the European Atlantic and Mediterranean waters, since natural sources of As in these areas seem to be more relevant than in North America where the threshold levels used for comparison were estimated (V. Besada, *pers. comm.*).

5.4.3 Overview of health condition

No correlations among any of the pollutants analysed and condition indices were observed at individual levels, though site differences were observed. Individuals from the Ebro Delta displayed slightly higher HSI indices than individuals from the other sampled sites, and females from Barcelona had the highest GSI values. In the latter case, it would seem to be related to the difference in size, whilst higher values of HSI could be the result of a better condition thanks to more favourable environmental conditions in that area. Bailey et al. (1986) described how the physical characteristics such as the sediment's particle size distribution and organic carbon content seemed to influence biological characteristics. For instance, muddy sediments are more suitable for the construction and maintenance of extensive burrow complexes and a significant correlation between areas with a finer composition, higher population densities and also bigger individuals has been observed in the Clyde Sea (Bailey et al., 1986; Campbell et al., 2009).

On the other hand, enlarged hepatopancreas can also be related to chronic toxic responses due to the increased contribution of biotransformation enzymes (Crespo and Solé, 2016). This hypothesis would only be partially supported by the determination of enzymatic activities since the only significant differences observed were the mean higher LDH activities in the Ebro Delta than other locations. Even though the variations observed could as well fall in the natural variability of the species (Antó et al., 2009), elevated LDH activities as a response to metal exposure have been demonstrated in fish (Vieira et al., 2009) and individuals in the Ebro Delta showed the highest levels of several of the heavy metals known to have an impact on this species (e.g. Zn, Mn, Co, Cu) (Eriksson et al., 2013).

Another significant correlation observed for enzymatic activities was that observed for the total load of fibres and a potential inhibition of CAT as well as an increased GST activity. This very same pattern of response was observed in gills of *Scrobicularia plana* exposed to polystyrene microplastics under controlled conditions (Ribeiro et al., 2017). In that study, the response observed, which was further coupled to changes in other biomarkers (acetylcholinesterase, lipid peroxidation and superoxide dismutase), was related to an oxidative stress response. Similar trends in these biomarkers have been correlated with plastics in studies with fish (Alomar et al., 2017), small crustaceans (Jeong et al., 2017, 2016), mussels (Avio et al., 2015; Paul-Pont et al., 2016), and nematodes (Lei et al., 2018).

All these findings suggest that plastics, in particular smaller particles, might induce the formation of reactive oxygen species in different groups of organisms and that assessing antioxidant enzymes might be suitable for environmental monitoring of plastic pollution (Suman et al., 2021). Martinelli et al. (2021) described the presence of plastic particles, mostly in the range between 50 and 100 µm, in the hepatopancreas. Given that the presence of larger ingested plastics might be likely correlated with the levels of smaller particles, especially considering the ability of the stomach in promoting the fragmentation of plastics (Cau et al., 2020), further studies would be needed to test whether the trends observed in enzymatic activities might actually be related to the presence of plastics in other tissues or in sizes not assessed in this study.

No relevant histological alterations were identified other than occasional findings of what looked like common host responses in crustaceans, probably involving haemocytes surrounding and encapsulating an unidentified element (Battistella et al., 1996). The most relevant though incidental finding was the observation of cyst-like structures in the intestinal submucosa with aggregations of elongated to ovoid yeast-like cells in their interior. These structures might resemble those described in edible crabs from the English Channel co-infected by a yeast-like organism and *Hematodinium* sp. (Stentiford et al., 2003). However, unlike it was described for crabs, other similar structures or free cells were not observed elsewhere (i.e., in the hepatopancreas sinuses or the haemolymph surrounding the hepatopancreatic tubules) nor were observed phagocytosed within haemocytes. Further investigation is currently being undertaken to better describe and characterise these structures and their potential etiological agent. It should also be noted that *Hematodinium* spp. or other parasitic dinoflagellates commonly found in other populations of the species (Stentiford and Shields, 2005) were not reported in individuals from the Mediterranean Sea. Even though there exist certain limitations in a description solely based on H&E stained preparations, heavily infected individuals, for which aggregations of dinoflagellates would appear in the hepatopancreatic sinuses and infiltrate into ovarian follicles were not observed (Field and Appleton, 1995; Stentiford and Neil, 2011). In broad terms, individuals did not show signs of pathological conditions compatible with exposure to pollutants and tissue organization matched those considered normal in other model species.

In conclusion, our results demonstrate that both plastic ingestion and heavy metal accumulation are common phenomena in the population of *N. norvegicus* from the NW Mediterranean Sea. Some correlations with enzymatic activities were also observed, though causation relationships can hardly be implied in field studies. In any case, a potential oxidative response to plastic ingestion leading to the increase in GST activity and the inhibition of CAT, and high LDH activities and HSI values that could be related to metal exposure were observed. Given the absence of significant pathological alterations in target organs as well as the absence of clear correlations between condition indices and the levels of contaminants described suggest an overall good health status. However, particularly high levels of As (above TET threshold levels) in sediments have been highlighted in the area near Barcelona and the Ebro Delta, and further research would be needed to better assess the potential impact at a community level. Monitoring programmes are also advised on the levels of As in the edible portion to ensure food safety. The simultaneous characterization of environmental and biological levels of plastic contamination has provided further evidence of the great potential of this species as an indicator species for plastic pollution in the environment. In broad terms, this multidisciplinary approach has proved a suitable approach for a general health assessment of *N. norvegicus* with particular regards to plastics and heavy metals.

Acknowledgements

We would like to thank Dr. Stentiford for his aid in the histological assessment. This study was supported by the Spanish Ministry of Science, Innovation and Universities project "PLASMAR" (RTI2018-094806-B-100) and by the Catalan Department of Agriculture, Livestock, Fisheries and Food (European Maritime and Fisheries Fund (EMFF)) project "SOMPESCA" (ARP059/19/00003). We thank all fishermen from commercial fishing vessels involved in the "PLASMAR" and "SOMPESCA" projects. Carreras-Colom benefits from an FPU PhD student grant from the Spanish Ministry of Science, Innovation and Universities (FPU16/03430).

References

Aebi, H., 1974. Methods of enzymatic analysis, Bergamyer. ed. Academic London.

Ahearn, G.A., Mandal, P.K., Mandal, A., 2004. Mechanisms of heavy-metal sequestration and detoxification in crustaceans: A review. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 174, 439–452. https://doi.org/10.1007/s00360-004-0438-0

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1–10. https://doi.org/10.1016/j.marenvres.2016.01.005

Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., Deudero, S., 2017. Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for causing oxidative stress. Environ. Res. 159, 135–142. https://doi.org/10.1016/j.envres.2017.07.043

Anastasopoulou, A., Fortibuoni, T., 2019. Impact of Plastic Pollution on Marine Life in the Mediterranean Sea, in: The Handbook of Environmental Chemistry. https://doi.org/10.1007/698_2019_421

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Antó, M., Arnau, S., Buti, E., Cortijo, V., Gutiérrez, E., Solé, M., 2009. Characterisation of integrated stress biomarkers in two deep-sea crustaceans, *Aristeus antennatus* and *Nephrops norvegicus*, from the NW fishing grounds of the Mediterranean sea. Ecotoxicol. Environ. Saf. 72, 1455–1462. https://doi.org/10.1016/j.ecoenv.2009.02.007

Avio, C.G., Cardelli, L.R., Gorbi, S., Pellegrini, D., Regoli, F., 2017. Microplastics pollution after the removal of the Costa Concordia wreck: First evidences from a biomonitoring case study. Environ. Pollut. 227, 207–214. https://doi.org/10.1016/j.envpol.2017.04.066

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., D'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222. https://doi.org/10.1016/j.envpol.2014.12.021

Bailey, N., Howard, F.G., Chapman, C.J., 1986. Clyde *Nephrops*: biology and fisheries. Proc. R. Soc. Edinburgh. Sect. B. Biol. Sci. 90, 501–518. https://doi.org/10.1017/s0269727000005194

Battistella, S., Bonivento, P., Amirante, G.A., 1996. Hemocytes and immunological reactions in crustaceans. Ital. J. Zool. 63, 337–343. https://doi.org/10.1080/11250009609356156

Becker, C., Cunningham, E.M., Dick, J.T.A., Eagling, L.E., Sigwart, J.D., 2018. A unified scale for female reproductive stages in the Norway lobster (*Nephrops norvegicus*): Evidence from macroscopic and microscopic characterization. J. Morphol. 279, 1700–1715. https://doi.org/10.1002/jmor.20852

Bell, T.A., Lightner, D. V, 1988. A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society, Baton Rouge, Louisiana.

Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. Front. Ecol. Environ. 15, 84–90. https://doi.org/10.1002/fee.1450

Bonanno, G., Orlando-Bonaca, M., 2018. Perspectives on using marine species as bioindicators of plastic pollution. Mar. Pollut. Bull. 137, 209–221. https://doi.org/10.1016/j.marpolbul.2018.10.018

Bradford, M., 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1006/abio.1976.9999

Burke, M.D., Mayer, R.T., 1974. Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Met Dispos 2, 583–588.

Campbell, N., Allan, L., Weetman, A., Dobby, H., 2009. Investigating the link between *Nephrops norvegicus* burrow density and sediment composition in Scottish waters. ICES J. Mar. Sci. 66, 2052–2059. https://doi.org/10.1093/icesjms/fsp176

Canals, M., Puig, P., de Madron, X.D., Heussner, S., Palanques, A., Fabres, J., 2006. Flushing submarine canyons. Nature 444, 354–357. https://doi.org/10.1038/nature05271

Canli, M., Furness, R.W., 1993. Heavy Metals in Tissues of the Norway Lobster *Nephrops norvegicus*: Effects of Sex, Size and Season. Chem. Ecol. 8, 19–32. https://doi.org/10.1080/02757549308035297

Carreras-Colom, E., Constenla, M., Soler-Membrives, A., Cartes, J.E., Baeza, M., Carrassón, M., 2020. A closer look at anthropogenic fiber ingestion in *Aristeus antennatus* in the NW Mediterranean Sea: Differences among years and locations and impact on health condition. Environ. Pollut. 263. https://doi.org/10.1016/j.envpol.2020.114567

Carreras-Colom, E., Constenla, M., Soler-Membrives, A., Cartes, J.E., Baeza, M., Padrós, F., Carrassón, M., 2018. Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*. Mar. Pollut. Bull. 133, 44–52. https://doi.org/10.1016/j.marpolbul.2018.05.012

Carson, H.S., 2013. The incidence of plastic ingestion by fishes: From the prey's perspective. Mar. Pollut. Bull. 74, 170–174. https://doi.org/10.1016/j.marpolbul.2013.07.008

Cau, A., Avio, C.G., Dessì, C., Follesa, M.C., Moccia, D., Regoli, F., Pusceddu, A., 2019. Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: Flagship species for deep-sea environments? Environ. Pollut. 255, 113107. https://doi.org/10.1016/j.envpol.2019.113107

Cau, A., Avio, C.G., Dessì, C., Moccia, D., Pusceddu, A., Regoli, F., Cannas, R., Follesa, M.C., 2020. Benthic Crustacean Digestion Can Modulate the Environmental Fate of Microplastics in the Deep Sea. Environ. Sci. Technol. 54, 4886–4892. https://doi.org/10.1021/acs.est.9b07705

Childress, J., Somero, G., 1990. Metabolic scaling: a new perspective based on scaling of glycolytic enzyme activities. Am Zool 30, 161–173.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528. https://doi.org/10.1038/srep04528

Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O.M., Narayanaswamy, B.E., 2017. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environ. Pollut. 231, 271–280. https://doi.org/10.1016/j.envpol.2017.08.026

Crespo, M., Solé, M., 2016. The use of juvenile *Solea solea* as sentinel in the marine platform of the Ebre Delta: in vitro interaction of emerging contaminants with the liver detoxification system. Environ. Sci. Pollut. Res. 23, 19229–19236. https://doi.org/10.1007/s11356-016-7146-7

de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the Western Mediterranean Sea. Mar. Pollut. Bull. 140, 523–535. https://doi.org/10.1016/j.marpolbul.2019.01.053

De Stephanis, R., Giménez, J., Carpinelli, E., Gutierrez-Exposito, C., Cañadas, A., 2013. As main meal for sperm whales: Plastics debris. Mar. Pollut. Bull. 69, 206–214. https://doi.org/10.1016/j.marpolbul.2013.01.033

De Wael, K., Baes, C., Lepot, L., Gason, F., 2011. On the frequency of occurrence of a peculiar polyester fibre type found in blue denim textiles. Sci. Justice 51, 154–162.

Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., Vethaak, A.D., 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Mar. Pollut. Bull. 98, 179–187. https://doi.org/10.1016/j.marpolbul.2015.06.051

EC, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance) 5–24.

EFSA, P. on F.A. and F., 2021. Safety assessment of titanium dioxide (E171) as a food additive. EFSA J. 19, e06585. https://doi.org/https://doi.org/10.2903/j.efsa.2021.658

Ellman, G., Courtney, K., Andres, V., Featherstone, R., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7, 88–95.

Eriksson, S.P., Hernroth, B., Baden, S.P., 2013. Stress Biology and Immunology in Nephrops norvegicus. Adv. Mar. Biol. Vol. 64 64, 149–200.

Feist, S.W., Stentiford, G.D., Kent, M.L., Ribeiro Santos, A., Lorance, P., 2015. Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean. Mar. Environ. Res. 106, 42–50. https://doi.org/10.1016/j.marenvres.2015.02.004

Ferrante, M., Napoli, S., Grasso, A., Zuccarello, P., Cristaldi, A., Copat, C., 2019. Systematic review of arsenic in fresh seafood from the Mediterranean Sea and European Atlantic coasts: A health risk assessment. Food Chem. Toxicol. 126, 322–331. https://doi.org/10.1016/j.fct.2019.01.010

Field, R., Appleton, P., 1995. A *Hematodinium*-like dinoflagellate infection of the Norway lobster *Nephrops norvegicus*: observations on pathology and progression of infection. Dis. Aquat. Organ. 22, 115–128. https://doi.org/10.3354/dao022115

Fischer, V., Elsner, N.O., Brenke, N., Schwabe, E., Brandt, A., 2015. Plastic pollution of the Kuril–Kamchatka Trench area (NW pacific). Deep Sea Res. Part II Top. Stud. Oceanogr. 111, 399–405. https://doi.org/10.1016/j.dsr2.2014.08.012

Fontcuberta, M., Calderon, J., Villalbí, J.R., Centrich, F., Portaña, S., Espelt, A., Duran, J., Nebot, M., 2011. Total and inorganic arsenic in marketed food and associated health risks for the Catalan (Spain) population. J. Agric. Food Chem. 59, 10013–10022. https://doi.org/10.1021/jf2013502

Fossi, M.C., Pedà, C., Ferrer, M.C., Tsangaris, C., Mascaró, C.A., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2017. Biondicators for monitoring marine litter ingestion and impacts on

Mediterranean biodiversity. Environ. Pollut. https://doi.org/10.1016/j.envpol.2017.11.019

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull. 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022

Galafassi, S., Nizzetto, L., Volta, P., 2019. Plastic sources: A survey across scientific and grey literature for their inventory and relative contribution to microplastics pollution in natural environments, with an emphasis on surface water. Sci. Total Environ. 693, 133499. https://doi.org/10.1016/j.scitotenv.2019.07.305

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-Transferases. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Habte, G., Choi, J.Y., Nho, E.Y., Oh, S.Y., Khan, N., Choi, H., Park, K.S., Kim, K.S., 2015. Determination of toxic heavy metal levels in commonly consumed species of shrimp and shellfish using ICP-MS/OES. Food Sci. Biotechnol. 24, 373–378. https://doi.org/10.1007/s10068-015-0049-4

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297

Hermsen, E., Pompe, R., Besseling, E., Koelmans, A.A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Mar. Pollut. Bull. 122, 253–258. https://doi.org/10.1016/j.marpolbul.2017.06.051

Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science (80-.). 293, 629–637. https://doi.org/10.1126/science.1059199

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/10.1126/science.1260352

Jeong, C., Kang, H., Lee, M.-C., Kim, D., Han, J., Hwang, D., Souissi, S., Lee, S.-J., Shin, K., Park, H.G., Lee, J., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana. Sci. Rep. 7, 41323. https://doi.org/10.1038/srep41323

Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., Hwang, U.K., Zhou, B., Souissi, S., Lee, S.J., Lee, J.S., 2016. Microplastic Size-Dependent Toxicity, Oxidative Stress Induction, and p-JNK and p-p38 Activation in the Monogonont Rotifer (*Brachionus koreanus*). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01441

Johnson, L.L., Anulacion, B.F., Arkoosh, M.R., Burrows, D.G., da Silva, D.A.M., Dietrich, J.P., Myers, M.S., Spromberg, J., Ylitalo, G.M., 2013. Effects of Legacy Persistent Organic Pollutants (POPs) in Fish-Current and Future Challenges, First Edit. ed, Fish Physiology. Elsevier Inc. https://doi.org/10.1016/B978-0-12-398254-4.00002-9

Johnson, M.L., Johnson, M., 2013. The ecology and biology of *Nephrops norvegicus*. Advances in Marine Biology, London, UK. https://doi.org/10.2307/3523

Jordana, E., Pinedo, S., Ballesteros, E., 2015. Macrobenthic assemblages, sediment characteristics and heavy metal concentrations in soft-bottom Ebre Delta bays (NW Mediterranean). Environ. Monit. Assess. 187, 71. https://doi.org/10.1007/s10661-015-4315-y

Kanhai, L.D.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Mar. Pollut. Bull. 115, 307–314. https://doi.org/10.1016/j.marpolbul.2016.12.025

Koenig, S., Fernández, P., Company, J.B., Huertas, D., Solé, M., 2013. Are deep-sea organisms dwelling within a submarine canyon more at risk from anthropogenic contamination than those from the adjacent open slope? A case study of Blanes canyon (NW Mediterranean). Prog. Oceanogr. 118, 249–259. https://doi.org/10.1016/j.pocean.2013.07.016

Lebreton, L.C.M., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 1–10. https://doi.org/10.1038/ncomms15611

Lefebvre, C., Saraux, C., Heitz, O., Nowaczyk, A., Bonnet, D., 2019. Microplastics FTIR characterisation and distribution in the water column and digestive tracts of small pelagic fish in the Gulf of Lions. Mar. Pollut. Bull. 142, 510–519. https://doi.org/10.1016/j.marpolbul.2019.03.025

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103

Liu, F., Vianello, A., Vollertsen, J., 2019. Retention of microplastics in sediments of urban and highway stormwater retention ponds. Environ. Pollut. 255, 113335. https://doi.org/10.1016/j.envpol.2019.113335

Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage. 19, 81–97. https://doi.org/10.1007/BF02472006

Loo, L.O., Pihl Baden, S., Ulmestrand, M., 1993. Suspension feeding in adult *Nephrops norvegicus* (L.) and *Homarus gammarus* (L.) (decapoda). Netherlands J. Sea Res. 31, 291–297. https://doi.org/10.1016/0077-7579(93)90029-R

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31. https://doi.org/10.1007/s002440010075

Martinelli, M., Gomiero, A., Guicciardi, S., Frapiccini, E., Strafella, P., Angelini, S., Domenichetti, F., Belardinelli, A., Colella, S., 2021. Preliminary results on the occurrence and anatomical distribution of microplastics in wild populations of *Nephrops norvegicus* from the Adriatic Sea. Environ. Pollut. 278, 116872. https://doi.org/10.1016/j.envpol.2021.116872

Masura, J., Baker, J., Foster, G., Arthur, C., 2015. Laboratory Methods for the Analysis of Microplastics in the Marine Environment. NOAA Mar. Debris Progr. Natl. 1–39.

Mateos-Cárdenas, A., O'Halloran, J., van Pelt, F.N.A.M., Jansen, M.A.K., 2021. Beyond plastic microbeads – Short-term feeding of cellulose and polyester microfibers to the freshwater amphipod *Gammarus duebeni*. Sci. Total Environ. 753, 141859. https://doi.org/10.1016/j.scitotenv.2020.141859

McGoran, A.R., Clark, P.F., Smith, B.D., Morritt, D., 2020. High prevalence of plastic ingestion by *Eriocheir sinensis* and *Carcinus maenas* (Crustacea: Decapoda: Brachyura) in the Thames Estuary. Environ. Pollut. 265, 114972. https://doi.org/10.1016/j.envpol.2020.114972

McGoran, A.R., Cowie, P.R., Clark, P.F., McEvoy, J.P., Morritt, D., 2018. Ingestion of plastic by fish: A comparison of Thames Estuary and Firth of Clyde populations. Mar. Pollut. Bull. 137, 12–23. https://doi.org/10.1016/j.marpolbul.2018.09.054

Mordecai, G., Tyler, P.A., Masson, D.G., Huvenne, V.A.I., 2011. Litter in submarine canyons off the west coast of Portugal. Deep Sea Res. Part II Top. Stud. Oceanogr. 58, 2489–2496. https://doi.org/10.1016/j.dsr2.2011.08.009

Mrosovsky, N., Ryan, G.D., James, M.C., 2009. Leatherback turtles: The menace of plastic. Mar. Pollut. Bull. 58, 287–289. https://doi.org/10.1016/j.marpolbul.2008.10.018

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175

Österblom, H., Jouffray, J.B., Folke, C., Rockström, J., 2017. Emergence of a global science–business initiative for ocean stewardship. Proc. Natl. Acad. Sci. U. S. A. 114, 9038–9043. https://doi.org/10.1073/pnas.1704453114

Palanques, A., López, L., Guillén, J., Puig, P., 2020. Trace metal variability controlled by hydrodynamic processes in a polluted inner shelf environment (Besòs prodelta, NW Mediterranean). Sci. Total Environ. 735, 139482. https://doi.org/10.1016/j.scitotenv.2020.139482

Palanques, A., Lopez, L., Guillén, J., Puig, P., Masqué, P., 2017. Decline of trace metal pollution in the bottom sediments of the Barcelona City continental shelf (NW Mediterranean). Sci. Total Environ. 579, 755–767. https://doi.org/10.1016/j.scitotenv.2016.11.031

Palanques, A., Masqué, P., Puig, P., Sanchez-Cabeza, J.A., Frignani, M., Alvisi, F., 2008. Anthropogenic trace metals in the sedimentary record of the Llobregat continental shelf and adjacent Foix Submarine Canyon (northwestern Mediterranean). Mar. Geol. 248, 213–227. https://doi.org/10.1016/j.margeo.2007.11.001

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737. https://doi.org/10.1016/j.envpol.2016.06.039

Pinedo, S., Jordana, E., Flagella, M.M., Ballesteros, E., 2014. Relationships between heavy metals contamination in shallow marine sediments with industrial and urban development in Catalonia (Northwestern Mediterranean Sea). Water. Air. Soil Pollut. 225.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When Microplastic Is Not Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass Macrophytodetritus. Environ. Sci. Technol. 49, 11158–11166.

Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in *Scrobicularia plana*. Mar. Pollut. Bull. 122, 379–391. https://doi.org/10.1016/j.marpolbul.2017.06.078

Rodríguez-Romeu, O., Constenla, M., Carrassón, M., Campoy-Quiles, M., Soler-Membrives, A., 2020. Are anthropogenic fibres a real problem for red mullets (*Mullus barbatus*) from the NW Mediterranean? Sci. Total Environ. 733, 139336. https://doi.org/10.1016/j.scitotenv.2020.139336

Rodríguez, E.M., Medesani, D.A., Fingerman, M., 2007. Endocrine disruption in crustaceans due to pollutants: A review. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 146, 661–671. https://doi.org/10.1016/j.cbpa.2006.04.030

Rotllant, G., Ribes, E., Company, J. baptista, Durfort, M., 2005. The ovarian maturation cycle of the norway lobster nephrops norvegicus (linnaeus, 1758) (crustacea, decapoda) from the western mediterranean sea. Invertebr. Reprod. Dev. 48, 161–169. https://doi.org/10.1080/07924259.2005.9652182

Ruiz-Orejón, L.F., Sardá, R., Ramis-Pujol, J., 2018. Now, you see me: High concentrations of floating plastic debris in the coastal waters of the Balearic Islands (Spain). Mar. Pollut. Bull. 133, 636–646. https://doi.org/10.1016/j.marpolbul.2018.06.010

Salvador Cesa, F., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings. Sci. Total Environ. 598, 1116–1129. https://doi.org/10.1016/j.scitotenv.2017.04.172

Sanchez-Cabeza, J.A., Masqué, P., Ani-Ragolta, I., Merino, J., Frignani, M., Alvisi, F., Palanques, A., Puig, P., 1999. Sediment accumulation rates in the southern Barcelona continental margin (NW Mediterranean Sea) derived from 210Pb and 137Cs chronology. Prog. Oceanogr. 44, 313–332. https://doi.org/10.1016/S0079-6611(99)00031-2

Sanchez-Vidal, A., Pasqual, C., Kerhervé, P., Calafat, A., Heussner, S., Palanques, A., Durrieu de Madron, X., Canals, M., Puig, P., 2008. Impact of dense shelf water cascading on the transfer of organic matter to the deep western Mediterranean basin. Geophys. Res. Lett. 35, L05605. https://doi.org/10.1029/2007GL032825

Sanchez-Vidal, A., Thompson, R.C., Canals, M., de Haan, W.P., 2018. The imprint of microfibres in southern European deep seas. PLoS One 13, e0207033. https://doi.org/10.1371/journal.pone.0207033

Santana, C.A. da S., Wieczorek, A.M., Browne, P., Graham, C.T., Power, A.M., 2020. Importance of suspended particulate organic matter in the diet of *Nephrops norvegicus* (Linnaeus, 1758). Sci. Rep. 10, 3387. https://doi.org/10.1038/s41598-020-60367-x

Satoh, T., Hosokawa, M., 2006. Structure, function and regulation of carboxylesterases. Chem. Biol. Interact. 162, 195–211. https://doi.org/10.1016/j.cbi.2006.07.001

Shields, J.D., Boyd, R.A., 2014. Atlas of Lobster Anatomy and Histology. Virginia Institute of Marine Science.

Simon-Sánchez, L., Grelaud, M., Garcia-Orellana, J., Ziveri, P., 2019. River Deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean). Sci. Total Environ. 687, 1186–1196. https://doi.org/10.1016/j.scitotenv.2019.06.168

Simon, M., van Alst, N., Vollertsen, J., 2018. Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. Water Res. 142, 1–9. https://doi.org/10.1016/j.watres.2018.05.019

Solé, M., Sanchez-Hernandez, J.C., 2018. Elucidating the importance of mussel carboxylesterase activity as exposure biomarker of environmental contaminants of current concern: An in vitro study. Ecol. Indic. 85, 432–439. https://doi.org/10.1016/j.ecolind.2017.10.046

Stentiford, G., Evans, M., Bateman, K., Feist, S., 2003. Co-infection by a yeast-like organism in *Hematodinium*-infected European edible crabs *Cancer pagurus* and velvet swimming crabs *Necora puber* from the English Channel. Dis. Aquat. Organ. 54, 195–202. https://doi.org/10.3354/dao054195

Stentiford, G.D., Neil, D.M., 2011. Diseases of *Nephrops* and *Metanephrops*: A review. J. Invertebr. Pathol. 106, 92–109. https://doi.org/10.1016/j.jip.2010.09.017

Stentiford, G.D., Shields, J.D., 2005. A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. Dis. Aquat. Organ. 66, 47–70. https://doi.org/10.3354/dao066047

Suaria, G., Achtypi, A., Perold, V., Lee, J.R., Pierucci, A., Bornman, T.G., Aliani, S., Ryan, P.G., 2020. Microfibers in oceanic surface waters: A global characterization. Sci. Adv. 6, eaay8493. https://doi.org/10.1126/sciadv.aay8493

Suman, K.H., Haque, M.N., Uddin, M.J., Begum, M.S., Sikder, M.H., 2021. Toxicity and biomarkers of micro-plastic in aquatic environment: a review. Biomarkers 26, 13–25. https://doi.org/10.1080/1354750X.2020.1863470

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, J., John, A.W.G., McGonigie, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science (80-.). 304, 838. https://doi.org/10.1126/science.1094559

Vassault, A., 1983. Lactate dehydrogenase, in: HO, B. (Ed.), Methods of Enzymatic Analysis, Vol III. Enzymes: Oxireductases Transferases. Academic Press, New York, pp. 118–126.

Vieira, L.R., Gravato, C., Soares, A.M.V.M., Morgado, F., Guilhermino, L., 2009. Acute effects of copper and mercury on the estuarine fish *Pomatoschistus microps*: Linking biomarkers to behaviour. Chemosphere 76, 1416–1427. https://doi.org/10.1016/j.chemosphere.2009.06.005

Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of Plastic Microfibers by the Crab *Carcinus maenas* and Its Effect on Food Consumption and Energy Balance. Environ. Sci. Technol. 49, 14597–14604. https://doi.org/10.1021/acs.est.5b04026

Welden, N.A.C., Cowie, P.R., 2016a. Environment and gut morphology influence microplastic retention in langoustine, *Nephrops norvegicus*. Environ. Pollut. 214, 859–865. https://doi.org/10.1016/j.envpol.2016.03.067

Welden, N.A.C., Cowie, P.R., 2016b. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ. Pollut. 218, 895–900. https://doi.org/10.1016/j.envpol.2016.08.020

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031

CHAPTER 6 An affordable method for monitoring plastic ingestion in *Nephrops norvegicus* (Linnaeus, 1758) and implementation on wide temporal and geographical scale comparisons

6 An affordable method for monitoring plastic ingestion in *Nephrops norvegicus* (Linnaeus, 1758) and implementation on wide temporal and geographical scale comparisons

Ester Carreras-Colom[a], Joan E. Cartes[b], Maria Constenla[a], Natalie A. Welden[c], Anna Soler-Membrives[a], Maite Carrassón[a]*

[a] *Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain*
[b] *Institut de Ciències del Mar (ICM-CSIC), Psg. Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain*
[c] *University of Glasgow, School of Interdisciplinary Studies, Dumfries DG1 4ZL, Scotland*

*Corresponding author: maite.carrason@uab.cat

SUMMARY

Although the ingestion of plastics has been reported in a wide variety of organisms, there remains a lack of knowledge regarding the extent of spatial and temporal gradients and no consensus concerning the definition of monitor species for benthic marine environments. The present study describes and compares plastic presence in stomach contents of several populations of Nephrops norvegicus across its European distribution by using two different approaches. First, plastic presence and load are described in detail, including fibre abundance and total fibre length, size distribution and polymer composition. Second, the prevalence of tangled balls of fibres is used as a proxy for total plastic fibre ingestion. Overall, locations with increased levels of plastic ingestion (e.g., the Gulf of Cadiz, N Barcelona and, partially, the Clyde Sea) also demonstrated higher levels of prevalence of balls (up to 30% of the individuals), compared to other locations with lower values of plastic ingestion and even the complete absence of balls (e.g., the Ebro Delta area, the NW Iberian margin). Differences could not be attributed to differences in diet composition and probably reflect environmental differences. As a result, observation of the prevalence of tangled balls of fibres, particularly in the well-studied Nephrops norvegicus, is proposed as an affordable, cost-effective and easy to implement indicator of plastic ingestion in benthic organisms and poses an excellent opportunity for established monitoring programs.

6.1 Introduction

Although large-scale production of plastics began in the 1950s and the first recordings of plastic debris in marine surface waters date back to the early 1970s (Carpenter and Smith, 1972), plastic pollution as a threat to marine ecosystems was largely ignored for many years up until recently (GESAMP, 2015). After two decades of exponential increase in research studies focused on this topic, plastic pollution is now widely recognised as a critical environmental concern by the public, scientific communities and policymakers. During the past decade, scientists have focused on describing the spread of plastic pollution, systematically reporting the presence of plastics in a wide range of environments as well as the digestive tracts of marine organisms (Li et al., 2016; Lusher et al., 2017; Provencher et al., 2019), as well as studying their potential impact through a broad range of laboratory and experimental studies (Foley et al., 2018; Jacob et al., 2020). Among plastic debris, microplastics, defined as synthetic solid particles of varied shapes and sizes ranging between 1 µm and either 1 or 5 mm (Frias and Nash, 2019; Hartmann et al., 2019), have received particular attention since they are perceived as a greater risk among marine biologists because of their ubiquity, persistence and size that facilitates ingestion by biota (Backhaus and Wagner, 2020).

As in any emerging field, recent efforts have also been directed to the adoption of standard methods and monitoring guides, leading to rapid growth in the publishing rate of methodological studies, reviews and guidelines (Cowger et al., 2020; Miller et al., 2017; Provencher et al., 2017; Rocha-Santos and Duarte, 2015). However, we are still missing a broad consensus on cost-effective protocols for monitoring microplastic litter and an adequate prioritization of target species and environments necessary to lay the foundations of an appropriate monitoring network.

In European waters, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) includes marine litter ingestion by biota as one of their descriptors for assessing the good environmental status (D10C3). Member States are charged with establishing a list of species to be assessed for plastic ingestion, which contrasts with the will of consistent criteria and methodologies across the European Union. At the regional level, litter ingestion by northern fulmars (*Fulmarus glacialis*) has been used in the OSPAR Common Indicator Assessment

(EcoQ0) since 2007 in the North Sea, and the ingestion of litter by sea turtles has been recently added as an indicator for the Bay of Biscay and Iberian coast (Decision of the Committee on Environmental Impacts of Human Activities). Similarly, in the Mediterranean, the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coasts and Related Assessment Criteria (implementation of the Regional Plan on Marine Litter Management in the Mediterranean, UN Environment/Mediterranean Action Plan) has selected the most common marine turtle species, *Caretta caretta*, as target species for marine litter ingestion (IMAP Candidate Indicator 24). However, limited opportunities to analyse plastic ingestion in these species exist for obvious reasons (level of legal protection/conservation status, scarcity of deceased individuals and dependence on the collection of faeces during the breeding season – in fulmars – or from by-caught individuals – in turtles). Moreover, given their migratory behaviour, they can provide information on plastic ingestion on large spatial and temporal scales but are unlikely to reflect local levels of plastic pollution (Bonanno and Orlando-Bonaca, 2018).

On the other side, mussels (*Mytilus edulis* and *M. galloprovincialis*) have been long and extensively used in monitoring programmes because of their sessile lifestyle, wide distribution and filtering capacity that maximises the exposure and uptake of chemical pollutants in the environment. Several studies have proved their potential usefulness in monitoring microplastics (J. Li et al., 2016; Phuong et al., 2018), though the size range of particles they can ingest is limited, they can only reflect pollution levels of highly coastal areas, and sample processing is generally complex (digestion of whole tissues and high-resolution identification methods needed). Given the constraints these species represent for plastic monitoring and considering the diverse suite of contaminants that plastics and microplastics are (Rochman et al., 2019), the use of multiple bioindicator species with different characteristics might be a more practical approach. Nektobenthic and pelagic fishes (*Boops boops* and *Scomber* sp., respectively) and fish with great commercial interest (*Mullus* sp., *Trigla* sp., *Dicentrarchus labrax*) have been suggested as potential candidates thanks to their high availability and reported values of plastic ingestion (UNEP/MAP SPA/RAC, 2018). Cau et al. (2019) recently discussed the potential use of the crustaceans *Aristeus antennatus* and *Nephrops norvegicus* as flagship species for plastic contamination in the deep-sea, given both their ecological relevance and value in fish markets and local communities. Both crustaceans but especially *N. norvegicus,* also accomplish a valuable feature to seek in a good monitor species, a wide geographical and depth distribution that may allow for multi-scale comparisons of plastic ingestion data from different locations.

The Norway lobster *Nephrops norvegicus* (Linnaeus, 1958) was one of the first marine species for which plastic ingestion was accurately reported. In 2011, Murray and Cowie thoroughly described plastic contamination in individuals from the Clyde Sea and already highlighted the potentially threatening presence of fibres (a prevalence of 83%), some of them tightly tangled into balls. However, the presence of apparently synthetic items had already been noticed by Bailey et al. (1986) during their thorough study of the ecology and biology of langoustines in the Clyde. Similarly, the presence of strands recalling nylon threads, presumably from fishing gears, was noted by Cristo and Cartes (1998) during their comparative study of the diet of populations from the Mediterranean and adjacent Atlantic shelf. During the past decade, other works have continued to report worrying values of plastic ingestion again in the Firth of Clyde (Welden and Cowie, 2016a) as well as from neighbouring Irish waters (Hara et al., 2020) and to a lesser extent in the Sardinian coast (Cau et al., 2019).

The occurrence of tangled balls of fibres seems most likely related to the crustaceans digestive system morphology and functioning that tangles plastic fibres rather than breaking them down (Welden and Cowie, 2016b). This has been pointed out as an aggravating factor since they may increase the percentage of foregut occupied by non-nutritional elements causing a false satiation effect and increasing the retention time of plastics (Welden and Cowie, 2016b). Prolonged experimental exposures to synthetic fibres through the diet have led to a significant decrease in the feeding rate and ultimately to a reduction in body condition (Welden and Cowie, 2016b). On the other side, the increase in size as a result of the entanglement of fibres might ease the determination of plastic presence during screening processes of stomach contents as they can be directly identified with no need for complex procedures (e.g., digestion, density-separation, filtration, staining methods). Therefore, they pose an excellent opportunity for routine monitoring networks where fast and cheap methods are needed given the severe resource restraints that environmental management faces.

The aim of this study is to propose the use of the prevalence of tangled balls of fibres as an easy and affordable indicator for plastic ingestion monitoring in the flagship species

Nephrops norvegicus. To achieve this aim, we undertook a comparison of two measures of plastic quantification from wild-caught individuals from across the species range, including from areas where plastic ingestion has never been described in this species or from past periods (1990-1992). Quantification methods include a detailed description of plastic ingestion and an extended comparison of the prevalence of tangled fibres (balls). Differences in diet composition among populations and their potential influence over plastic ingestion levels are also discussed as potential drivers of plastic uptake.

6.2 Materials and methods

6.2.1 Detailed description of plastic ingestion

Individuals of *Nephrops norvegicus* were collected from several locations across its European distribution, mainly on board of Research Vessels but also in collaboration with commercial fishing vessels in the framework of different research projects. All samples for the detailed description of plastic ingestion were collected in late spring or summer, from common fishing grounds at depths around 70 m (Clyde Sea) and 200 and 560 m (Gulf of Cadiz and Balearic Sea). In some locations, more than one sampling was performed (Fig. 1, Table 1A). Adult individuals from the Clyde Sea and the Gulf of Cadiz were immediately frozen or dissected and the stomach stored in ethanol. Adult individuals from the Balearic Sea were immediately fixed in Davidson's fixative (72 h) and then transferred and stored in ethanol. Cephalothorax length (CL, in mm), total weight (TW, in g), sex and carapace hardness (hard, soft or jelly) were recorded. The moulting stage was determined based on carapace hardness (before fixation); that is, individuals with hard carapaces were considered to be at the intermoult stage, jelly ones were considered post-moult, and soft ones to either be in early intermoult periods or a post-moult stage (Milligan et al., 2009).

Once in the laboratory, stomach contents were visually inspected under a stereomicroscope (40x) for plastics. Any suspected items were separated, classified as fibres (on the basis that one dimension was more than three times the other) or other-shaped items and observed through light microscopy (100-200x). Items with cellular or organic structures (e. g. ornamentation or segmentation) were discarded. Images were taken for the remaining items from which measures were recorded (minimum and maximum diameter, and total area for fragments, diameter and total length for fibres) using the AutoCAD 2018 software (Autodesk, Inc).

Fibres were further categorised (magnification 400x used) according to their visual appearance following predefined criteria (Carreras-Colom et al., 2020; Rodríguez-Romeu et al., 2020; Zhu et al., 2019) into six categories (Supplementary Material Table S1). All fragments and a subsample (ca. 5%) of each of the fibre categories were analysed through FTIR with a Tensor 27 FTIR spectrometer (Bruker Optik GbmH) equipped with a diamond attenuated total reflectance (ATR) unit (16 scans · cm^{-1}). The resulting spectra were treated (baseline corrections, peak normalization and selection of characteristic band, i.e. 860-1800 and 2800-3400 cm^{-1}) and then matched with personal and reference databases (Carreras-Colom et al., 2018; Primpke et al., 2018) using Spectragryph v1.2.15 (Menges, 2021). Spectra matching with a $QI \geq 0.7$ were accepted; those between $0.55 \leq QI < 0.7$ were further explored visually to assess matching representative peaks.

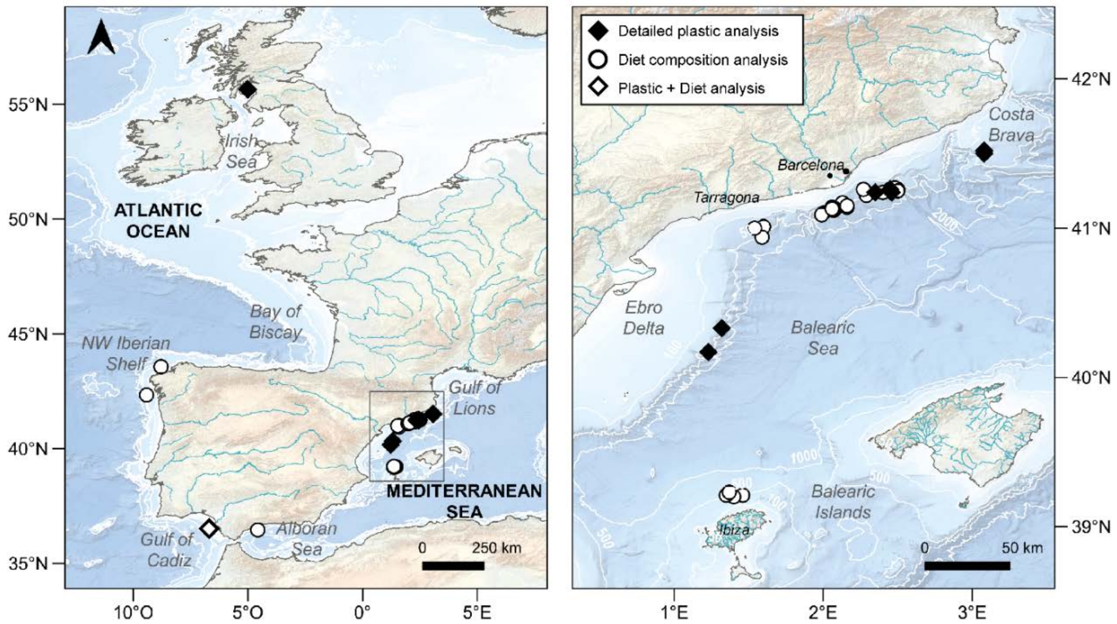


Fig. 1. Sampling locations on individuals of *Nephrops norvegicus* analysed for the presence of plastics (black diamonds), diet studies (white dots) and both (white diamond). Coordinate system used: WGS 84. Base maps retrieved from Web Map Servers: bathymetry (EMODnet Digital Bathymetry Consortium), depth contours (Instituto Español de Oceanografía, 2016), rivers (European Environment Agency, 2018).

Table 1. Sampling data (location, coordinates, mean depth (d) and date of the hauls) and prevalence of plastics in individuals of *Nephrops norvegicus* analysed for (**A**) the detailed description of plastic ingestion and (**B**) fast screening of the prevalence of balls and diet analysis. *n*: number of individuals analysed. R: items other than fibre-shaped ones; F: fibres of all types (isolated or tangled); B: aggregations of tangled fibres. Superscript numbers indicate that [(1)] material obtained from the same sampling was used for different purposes and [(2)] that the same location was targeted in different periods. Different superscript letters indicate significant differences among samplings.

Sampling data						*n*	% R	% F	% B
Location		Latitude	Longitude	*d*	Date				
A. Detailed description of plastic ingestion									
Clyde Sea	CS1	55˚ 39.54' N	5˚ 00.67' W	70	May 2019	30	3.3	56.7 [a]	3.3 [a]
	CS2				August 2019	30	0	83.3 [b]	23.3 [b]
Gulf of Cadiz	GC	36˚ 30.40' N	6˚ 41.30' W	325	May 2017	24[1]	0	83.3 [ab]	33.3 [b]
Balearic Sea									
Costa Brava	CB1	41˚ 31.26' N	3˚ 05.03' E	327	July 2018	20	0	75.0 [ab]	5.0 [a]
	CB2	41˚ 29.72' N	3˚ 04.85' E	355	July 2019	20	0	75.0 [ab]	20.0 [ab]
N Barcelona[2]	NB0	41˚ 14.49' N	2˚ 27.38' E	560	July 2007	20	0	90.0 [ab]	30.0 [b]
	NB1	41˚ 14.30' N	2˚ 21.23' E	380	July 2018	20	5.0	85.0 [ab]	35.0 [b]
	NB2	41˚ 15.64' N	2˚ 26.72' E	380	July 2019	20	5.0	85.0 [ab]	20.0 [ab]
Ebro Delta	ED1	40˚ 19.96' N	1˚ 19.23' E	200	September 2018	20	0	70.0 [ab]	0
	ED2	40˚ 09.94' N	1˚ 13.80' E	230	July 2019	20	0	95.0 [b]	0
B. Prevalence of balls and analysis of diet composition									
NW Iberian margin		42˚ 20.10' N	9˚ 25.00' W	95	September 2004	51	0		0
		43˚ 34.40' N	8˚ 47.20' W	350	September 2004	9	0		0
Gulf of Cadiz		36˚ 30.40' N	6˚ 41.30' W	325	May 2017	32+24[1]	9.4		30.3
Alboran Sea		36˚ 27.33' N	4˚ 34.16' E	225	May 2017	10	0		0
Balearic Sea									
N Barcelona[2]		41° 15.76' N	2° 27.30' E	430	January 1990	20	0		0
		41° 15.76' N	2° 27.30' E	430	September 1990	14	0		0
		41° 15.80' N	2° 28.13' E	428	December 1990	31	0		0
		41° 15.80' N	2° 28.13' E	428	February 1991	23	0		0
		41° 15.76' N	2° 29.75' E	430	March 1991	42	0		0
		41° 14.92' N	2° 16.54' E	275	April 1991	16	0		0
		41° 13.49' N	2° 17.60' E	347	April 1991	15	0		0
		41° 14.24' N	2° 24.29' E	380	November 1991	9	0		0
		41° 15.90' N	2° 28.72' E	424	November 1991	10	20.0		0
		41° 15.80' N	2° 28.13' E	428	February 1992	15	0		0
		41° 14.88' N	2° 29.71' E	504	April 1992	25	8.0		0
S Barcelona		41° 08.50' N	2° 03.69' E	425	November 1990	34	8.8		8.8
		41° 08.13' N	2° 03.49' E	390	April 1991	15	6.7		13.3
		41° 07.70' N	2° 04.42' E	457	April 1991	37	0		5.4
		41° 05.61' N	1° 59.64' E	395	May 1991	31	0		0
		41° 08.55' N	2° 09.50' E	427	November 1991	18	0		5.6
		41° 07.45' N	2° 04.20' E	355	March 1992	14	0		28.6
		41° 08.08' N	2° 03.54' E	422	July 1992	17	11.8		5.9
Tarragona		41° 00.15' N	1° 34.30' E	334	May 1992	24	0		0
		40° 56.60' N	1° 35.60' E	532	May 1992	5	0		0
Ibiza		39° 13.05' N	1° 21.50' E	525	May 1992	19	0		0
		39° 12.35' N	1° 25.75' E	456	May 1992	45	0		0

For each individual, the total load of synthetic fibres was estimated by the sum of fibres (fibre abundance) and the sum of lengths of fibres (total fibre length). These parameters were also calculated according to the fibre size (only abundance) and the polymer composition.

6.2.2 Extended data on the prevalence of balls and analysis of diet composition

A second analysis, including the determination of the prevalence of balls and the analysis of diet composition, was conducted on additional adult individuals sampled from the NW Iberian margin (September 2004), the Gulf of Cadiz (May 2017), the Alboran Sea (May 2017), and the Balearic Sea (throughout the years 1900-1992) at depths ranging between 95 and 504 m (Fig. 1, Table 1B). Size (CL, in mm), total weight (TW, in g) and moulting stage were recorded before dissection. Stomach contents were observed under the stereomicroscope and the presence of aggregations of tangled fibres (balls) and potentially synthetic fragments (> 1 mm) was recorded.

Prey was identified to the taxonomic level of group or family. Stomach contents were also weighed (CW, 0.1 mg). Since prey items were often not possible to weigh directly, their weight was estimated after the percentage volume occupied in stomach contents using the subjective points method (Swynnerton and Worthington, 1940), a methodology used in previous work on crustaceans diet (Cartes, 1994). Shannon-Wiener index (H′) (Shannon and Weaver, 1948) and Evenness (J′) (Pielou, 1975) were used to calculate trophic (diet) diversity. Feeding intensity was estimated through stomach fullness (F) as follows $F=CW/TW\times100$, in wet weight. In addition, prey items were classified into three ecological categories based on their habitat, swimming capacity and most likely location on the water column when ingested: planktonic-hyperbenthic (siphonophores, cephalopods, euphausiids, hyperiid amphipods, copepods, mysids, decapod crustaceans (Pasiphaeidae and Sergestidae), and myctophid fish), epibenthic (porifers, cnidarians (Hydrozoa), gastropods, pteropods, scaphopods, polychaetes (Serpulidae and Aphroditidae), brachyurs, decapod crustaceans (*N. norvegicus*), gammarid amphipods, isopods and fish remains), and endobenthic (foraminiferans, sipunculids, bivalves, polychaetes (*Glycera* sp. and Eunicidae), decapod crustaceans (Alpheus glaber), cumaceans and tanaids). The contribution (in percentage) of each category to the diet of each individual was calculated.

6.2.3 Quality assurance and quality control (QA/QC)

Dissection of individuals for the detailed analysis of plastics (section 2.1.; see Table 1A for details) was conducted in a safety cabinet when possible and screening of stomach contents was performed using an isolation device for the stereoscope (similarly to that used by Torre et al., 2016). All material used (scissors, tweezers and Petri dishes) were rinsed three times with filtered water (50 µm metal sieve) before use. To check for potential airborne contamination clean Petri dishes filled with filtered water were placed as close to the samples being screened as possible. Any fibres found in the stomach contents matching the characteristics of those found in the controls were discarded. That is colourless wrinkled fibres (resembling cellulosic fibres) placed on top of the water surface that also appeared not mixed with stomach contents nor covered with organic matter in the screening samples. No other corrections were performed.

During the analysis of diet composition (section 2.2.; see Table 1B) no strong specific measures were taken to prevent airborne contamination. Only the percentage of balls and macro-sized fragments was recorded in these examinations, and these categories are unlikely to come from airborne contamination during visual screening processes. Subsequently, no corrections of any type were performed.

6.2.4 Statistical analysis

Data was grouped according to location (Clyde Sea, NW Iberian margin, Gulf of Cadiz, Alboran Sea, Ebro Delta, Tarragona, S Barcelona, N Barcelona, Costa Brava and Ibiza) and sampling point (when different samplings were performed at the same location in different periods, e.g., in the Clyde Sea in May and August or in the Costa Brava in 2018 and 2019) and values of prevalence and mean load of plastics were calculated accordingly. Given their rare occurrence, fragments were not considered in the following analyses. Differences in the prevalence and mean load (in terms of abundance and total fibre length of all fibres and tangled fibres alone) among locations and samplings were tested using Chi-squared analysis and non-parametric Kruskal-Wallis tests, respectively. Post-hoc pairwise comparisons were performed using the Dunn's Multiple Comparison Test using Holm's method for p-adjustment. Differences in the size distribution of ingested fibres among locations were further examined by comparing the histograms of size frequencies by calculating the Kolmogorov-Smirnoff distance and testing for differences using bootstrapping. Differences in polymer composition of fibres among locations were tested through the PERmutational ANalysis Of Variance (PERMANOVA) on the Bray-Curtis distance-based resemblance matrices of square-root transformed data. The indicator value of each polymer was calculated using the Dufrene-Legendre indicator species analysis. Relation of the factors size, sex, moulting stage, stomach fullness and presence of balls with the prevalence and the total load of ingested plastics was also tested using Chi-squared analysis and by fitting generalized linear models using the logistic model for the prevalence and the poison distribution for the abundance and total length of fibres. Spearman's correlation among plastic descriptors and biological variables were calculated.

Analysis of diet composition was done independently of sex since there are no reported differences in feeding between males and females (Mytilineou et al., 1992). To prevent underestimation of soft prey items, nearly empty stomachs were not considered (gut volume <15%). Principal Coordinate Analysis (PCoA) techniques were used to visualize potential patterns in diet composition among locations and in relation to the presence/absence of balls. Differences were further tested using PERMANOVA analysis. First, analyses were performed using the complete diet matrix considering 14 prey groups (Foraminifera, Porifera, Sipuncula, Cnidaria, Bivalvia, Cephalopoda, Gastropoda, Other Mollusca, Polychaeta, Euphausiacea, Decapoda, Other Crustacea, Echinodermata and Osteichthyes). Then, analyses were performed considering the relative proportion of plankton-hyperbenthos, epibenthos, and endobenthos over the total of prey items identified. Square-root transformation was applied on the raw data matrix and Bray-Curtis distance-based resemblance matrix used. Differences in stomach fullness and diversity indices among locations and in relation to the presence/absence of balls were tested using Kruskal-Wallis tests.

All analyses were performed using the statistical software R, version 3.6.3, and using the packages "dunn.test" (non-parametric post-hoc comparisons), "Hmisc" (correlations), "vegan" (PERMANOVA and PCoA) and "labdsv" (indicator value). Significance level was set at $p < 0.05$ for all tests.

6.3 Results

6.3.1 Analysis of plastic ingestion

Detailed analysis of plastic prevalence and load

A total of 1738 fibres and 18 non-fibre-shaped particles (98.96 and 1.04% of the total, respectively) were identified from 224 adult *Nephrops norvegicus*. All non-fibre-shaped items were identified as synthetic: a rubber-like fragment made of polydimethylsiloxane (silicone), a thin and threaded laminate of polyethylene and fifteen sticky thin film-like particles of polychloroprene (a component of neoprene) (Fig. 2). Polymer identification from a subsample of fibres (6.26%) revealed that over 96% were of synthetic composition, being the residual 4% representatives of the same category, associated to cellulose (Supplementary Material Table S1, category F). Five synthetic polymers were identified in fibres: polyethylene terephthalate (polyester, PET), polyamide (PA), acrylonitrile (acrylic, Acr), polypropylene (PP) and polyethylene (PE). The accurate visual approach to categorisation of fibres was considered appropriate since items classified into the same visual category were identified as the same polymer in > 75% of the cases (Supplementary Material Table S1). Results of polymer identification on predefined categories of fibres were therefore used to define the most likely polymer composition of all fibres not analysed by means of FTIR. Cellulosic fibres were estimated to account for less than 2.5% of all fibres identified, with lengths ranging between 0.47 and 5.36 mm and mean values < 0.6 mm · ind^{-1}. These cellulosic fibres were not taken into account in further analysis.

Overall, 77.8% of the individuals were observed to have at least one synthetic fibre and 12.5% also showed aggregations of more than five tangled fibres (balls) (Table 1A). Only three individuals (1.3%) were seen to contain particles other than fibres. The proportion of individuals with fibres, which ranged between 56.7 and 95.0%, did not significantly vary among locations ($p > 0.05$), neither did the prevalence of balls ($p > 0.05$), yet it should be noted that none of the individuals sampled from the Ebro Delta area were seen with a ball in their stomach (not included in the former analysis) (Table 1A). Marginally significant differences were observed among samplings for the prevalence of fibres ($X^2 = 16.14$, df = 9, p = 0.06) with individuals sampled from the Clyde Sea in May having a lower prevalence compared to those sampled in August. Pairwise comparisons in the prevalence of balls among samplings also revealed significant differences, pointing out the smaller prevalence of balls also in the May sampled individuals from the Clyde Sea (3.3%) compared to those sampled in August in the same area (23.3%) or from the Gulf of Cadiz (33.3%) and N Barcelona (2007 and 2018 samplings; 30 and 35%, respectively) (Fisher's test; $p < 0.05$). The prevalence of balls in the Gulf of Cadiz also differed significantly from that of individuals from the Costa Brava in 2018 (5%) (Table 1A).

The number of synthetic fibres identified in the stomach contents of a single individual ranged between 0 and 75, with an average value of 7.60 ± 12.01 fibres · ind^{-1}. Significant differences were found when comparing mean values among locations (Kruskal-Wallis $X^2 = 15.21$, df = 4, $p = 0.004$) and samplings (Kruskal-Wallis $X^2 = 32.76$, df = 9, $p < 0.001$) (Table 2). The highest fibre abundance was seen in langoustines from the Gulf of Cadiz, followed by individuals from the August sampling in the Clyde Sea and N Barcelona (all three samplings). In contraposition, lower values were found for the Ebro Delta and Costa Brava samplings, and the May sampling in the Clyde Sea. Similar trends were seen for the total fibre length among locations (Kruskal-Wallis $X^2 = 11.23$, df = 4, $p = 0.02$) and samplings (Kruskal-Wallis $X^2 = 33.01$, df = 9, $p < 0.001$) for which also higher values were found in the Gulf of Cadiz and N Barcelona samplings compared to the Costa Brava and Ebro Delta locations, particularly in those individuals sampled in 2018 (Fig. 3A, Table 2). Post-hoc comparisons revealed that differences among samplings from each location were not significant except for the Clyde Sea, for which a decreased fibre abundance and total load was observed in May compared to August, and in the Ebro Delta where the mean abundance of fibres was higher in 2019 compared to 2018.

Diameters of fibres ranged between 0.013 and 0.22 mm, with mean values of 0.03 mm, and lengths varied between 0.1 and 44.7 mm. The most abundant fibres were those with lengths comprised between 1 and 10 mm, classified as meso-plastics (Hartmann et al., 2019), and corresponding to more than 60% of the fibres identified in every location. Micro-sized fibres (0.2-1 mm) were highly variable among locations (9.0-35.7%) and were always more abundant than macro-sized fibres (>10 mm), which only contributed to more than 5% of the fibres identified in August's Clyde Sea and Ebro Delta's samplings (Table 2). Kolmogorov-Smirnoff test on the fibre size distribution revealed significant differences among locations with individuals from the Costa Brava having a shifted distribution towards longer fibres compared to those from the Clyde Sea (K-S; $D = 0.1631$, $p = 0.0025$) or N Barcelona

(K-S; D = 0.173, $p < 0.001$), especially if only tangled fibres were considered (Supplementary Material Fig. S1). Considering fibres from all locations together, significant differences were observed between the size distribution of fibres found not tangled into balls (isolated) and those tangled in balls, with the latter having a greater proportion of long fibres (K-S; D = 0.159, $p < 0.001$).

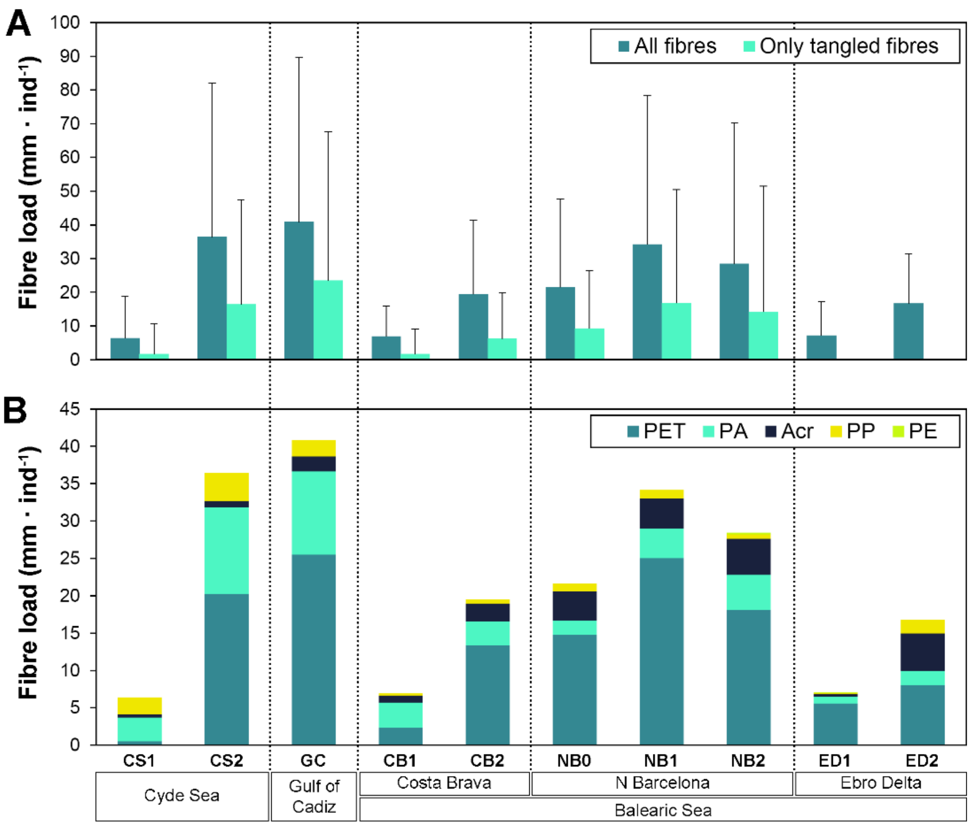


Fig. 3. Mean values of fibre load per individual found in stomach contents of *Nephrops norvegicus* sampled from several locations (Clyde Sea, Gulf of Cadiz, Costa Brava, N Barcelona and Ebro Delta) considering (**A**) the mean total fibre length per individual (mm · ind^{-1}) of all fibres and of tangled fibres alone and (**B**) the mean total fibre length per individual (mm · ind^{-1}) according to their polymer composition. PET: polyethylene terephthalate (polyester); PA: polyamide; Acr: acrylonitrile (Acrylic); PE: polyethylene; and PP: polypropylene). See Table 1 for details on sampling codes provided.

Several colours were identified, being the transparent fibres the most predominant (67.0%) followed by yellowed and brownish ones (8.9%), black (8.8%), red (7.1%) and blue (5.9%) fibres. Only in the Gulf of Cadiz one individual was observed with a high contribution of green fibres (11.6% of all fibres in that sampling location).

Table 2. Mean values of fibre load in terms of abundance (of all fibres, of tangled fibres only, and according to their length (FL): macro-, meso- and micro-) and total fibre length (of all fibres and of tangled fibres only) in stomach contents of *Nephrops norvegicus* sampled from the Clyde Sea (CS), the Gulf of Cadiz (GC) and several sites in the Balearic Sea (Costa Brava, CB, N Barcelona, NB, and Ebro Delta, ED). Different superscript letters among rows indicate significant differences among locations (uppercase letters) and samplings (lower case letters).

Sampling code	Abundance (n · ind^{-1})					Sum of lengths (mm · ind^{-1})	
	all fibres	tangled fibres	macrofibres (FL ≥ 10 mm)	mesofibres (1 ≥ FL > 10 mm)	microfibres (0.2 ≥ FL > 1 mm)	all fibres mm · ind^{-1}	tangled fibres mm · ind^{-1}
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Clyde Sea	**7.00 ± 11.90** ^{A}	**1.80 ± 5.32** ^{AC}	**0.37 ± 0.82** ^{A}	**5.30 ± 8.94** ^{A}	**1.48 ± 3.86** ^{AB}	**21.44 ± 36.42** ^{A}	**9.04 ± 23.84** ^{A}
CS1	2.77 ± 4.49 ^{a}	0.40 ± 2.19 ^{a}	0.10 ± 0.40 ^{a}	1.67 ± 2.89 ^{a}	1.00 ± 2.65 ^{a}	6.42 ± 12.40 ^{a}	1.64 ± 9.00 ^{a}
CS2	11.23 ± 15.20 ^{b}	3.20 ± 6.98 ^{b}	0.63 ± 1.03 ^{b}	8.63 ± 11.14 ^{b}	1.97 ± 4.77 ^{a}	36.46 ± 45.59 ^{b}	16.27 ± 31.01 ^{b}
Gulf of Cadiz	**13.08 ± 13.49** ^{B}	**6.08 ± 11.55** ^{B}	**0.54 ± 1.44** ^{A}	**10.42 ± 10.52** ^{B}	**2.25 ± 4.02** ^{A}	**40.79 ± 48.90** ^{B}	**23.50 ± 44.13** ^{B}
GC	13.08 ± 13.49 ^{b}	6.08 ± 11.55 ^{b}	0.54 ± 1.44 ^{ab}	10.42 ± 10.52 ^{b}	2.25 ± 4.02 ^{a}	40.79 ± 48.90 ^{b}	23.50 ± 44.13 ^{b}
Costa Brava	**4.35 ± 5.39** ^{A}	**0.90 ± 3.00** ^{A}	**0.10 ± 0.38** ^{A}	**3.88 ± 4.95** ^{A}	**0.45 ± 0.68** ^{AB}	**13.19 ± 17.72** ^{A}	**3.92 ± 11.11** ^{A}
CB1	2.50 ± 2.50 ^{ac}	0.25 ± 1.12 ^{ab}	0.10 ± 0.45 ^{a}	2.10 ± 2.22 ^{ac}	0.30 ± 0.47 ^{a}	6.93 ± 9.05 ^{ac}	1.64 ± 7.35 ^{a}
CB2	6.20 ± 6.80 ^{bc}	1.55 ± 4.05 ^{b}	0.10 ± 0.31 ^{ab}	5.50 ± 6.24 ^{bc}	0.60 ± 0.82 ^{a}	19.46 ± 21.90 ^{bc}	6.20 ± 13.73 ^{ab}
N Barcelona	**10.78 ± 16.19** ^{B}	**4.78 ± 12.79** ^{BC}	**0.20 ± 0.55** ^{A}	**8.10 ± 10.61** ^{B}	**2.58 ± 7.69** ^{AB}	**28.06 ± 37.97** ^{B}	**13.43 ± 30.30** ^{B}
NB0	9.40 ± 13.36 ^{b}	3.60 ± 8.36 ^{b}	0.10 ± 0.31 ^{a}	6.85 ± 9.31 ^{b}	2.45 ± 4.35 ^{a}	21.56 ± 26.18 ^{b}	9.24 ± 17.17 ^{b}
NB1	10.40 ± 14.08 ^{b}	4.35 ± 10.52 ^{b}	0.40 ± 0.82 ^{ab}	9.05 ± 11.67 ^{b}	0.95 ± 2.04 ^{a}	34.16 ± 44.20 ^{b}	16.84 ± 33.64 ^{ab}
NB2	12.55 ± 20.78 ^{b}	6.45 ± 17.96 ^{b}	0.10 ± 0.31 ^{ab}	8.15 ± 11.17 ^{b}	4.30 ± 12.43 ^{a}	28.45 ± 41.88 ^{b}	14.20 ± 37.32 ^{ab}
Ebro Delta	**3.68 ± 3.96** ^{A}	**0.00 ± 0.00**	**0.20 ± 0.46** ^{A}	**3.10 ± 3.48** ^{A}	**0.53 ± 0.82** ^{B}	**11.92 ± 13.36** ^{A}	**0.00 ± 0.00**
ED1	2.15 ± 2.76 ^{a}	0.00 ± 0.00	0.15 ± 0.37 ^{ab}	1.65 ± 1.84 ^{ac}	0.35 ± 0.81 ^{a}	7.11 ± 10.12 ^{ac}	0.00 ± 0.00
ED2	5.20 ± 4.44 ^{b}	0.00 ± 0.00	0.25 ± 0.55 ^{ab}	4.35 ± 4.18 ^{bc}	0.60 ± 0.68 ^{a}	16.73 ± 14.67 ^{bc}	0.00 ± 0.00

Multivariate analysis on polymer composition showed significant differences according to location and sampling (PERMANOVA; $p < 0.001$). In the Balearic Sea, in general, PET was the predominant polymer (~60%) followed by PA and Acr, whose contribution ranged between ~20-30% and ~15-20%, respectively (Fig. 3B, Supplementary Material Table S2). The greatest variations to this pattern were observed in individuals sampled in 2018 in the Costa Brava, where PET represented roughly one third of the total fibre load and PA took up to ~50%, and in 2019 in the Ebro Delta, where the relative proportion of Acr fibres raised to >30%, the highest value throughout the study. In both locations, the contribution of PP was also higher (~6%) compared to the rest of samplings in the Balearic Sea (<4%). Great similarities were found in the polymer composition of individuals sampled in August from the Clyde Sea and from the Gulf of Cadiz, with PET contributing to nearly half of the fibre load, being followed by PA (Fig. 3B; Supplementary Material Table S2). Finally, the highest contributions of PA (nearly 60%) and PP (~15%) were observed in individuals sampled in May from the Clyde Sea. Overall, PET and PA were identified as the polymers with the highest indicator value. PE was the least common polymer observed in fibres, representing less than 1% of the fibre load in the locations where it was identified (N Barcelona and Ebro Delta only).

Overall, general descriptors of fibre load (abundance and total length of fibres) were positively correlated among them ($rho > 0.6$), with the abundance of meso-plastics and total length of PA and PET fibres showing the highest correlation with the total load ($rho > 0.95$) (Fig. 4). The presence of balls was significantly related to higher levels of total plastic load in terms of abundance (GZM; $z = 37.46$, $p < 0.001$) and total length of fibres (GZM; $z=68.35$, $p < 0.001$).

Direct analysis of the prevalence of balls

From the total of 605 individuals screened for the presence of balls, only individuals from the Gulf of Cadiz and S Barcelona were observed with aggregations of tangled fibres, with values of prevalence ranging between 0 and 30.3% (Table 1B). Fragments were also found in these locations (prevalence ranging between 6.7 and 11.8% in those locations with positive individuals) and in two samplings performed in N Barcelona, with values of prevalence of 8 and 20% % (Table 1B). In particular, the samplings in S Barcelona showing the highest values of prevalence of balls (up to 28.6%) were those performed at the mouth of a submarine canyon (La Berenguera; S Barcelona). No fragments nor balls were recorded in individuals from the NW Iberian margin, the Alboran Sea, Ibiza or other areas along the slope of the Balearic Sea (N Barcelona and Tarragona). Available data does not allow for further analysis of monthly or yearly trends.

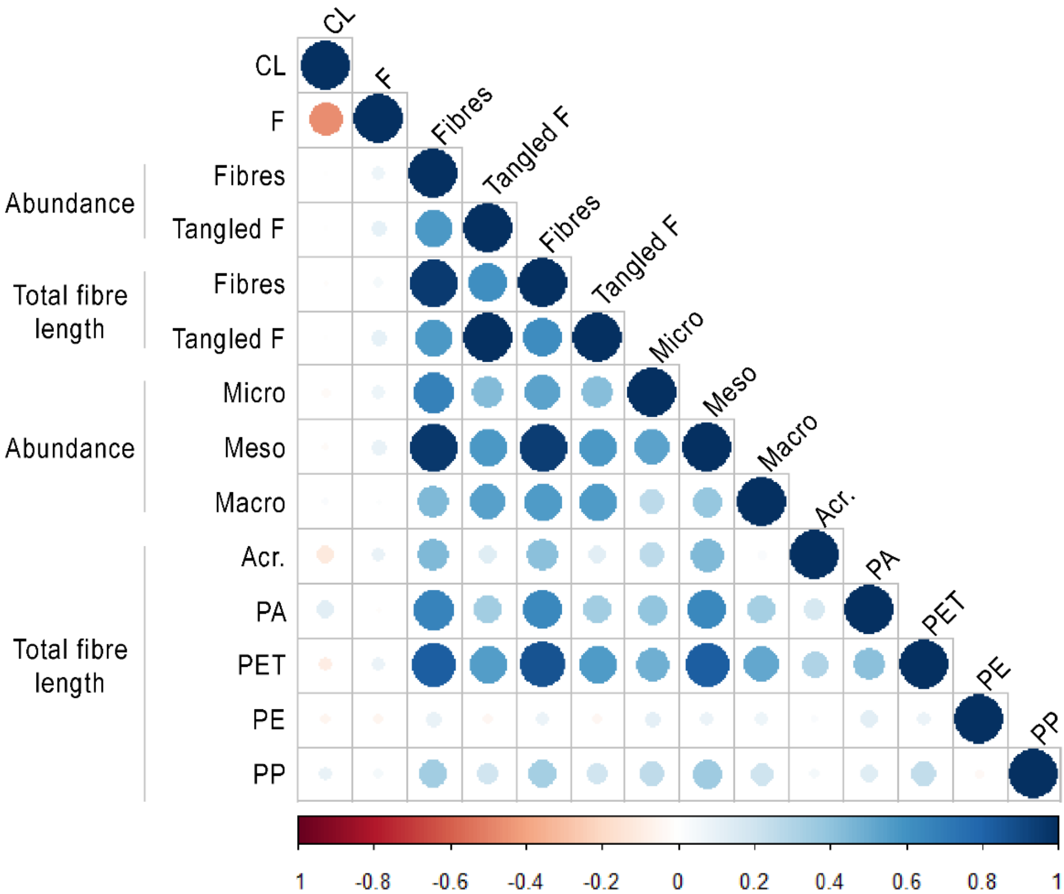


Fig. 4. Spearman's correlations among biological variables (CL: cephalothorax length; F: index of stomach fullness) and mean fibre load descriptors: abundance and total fibre length of all fibres and tangled fibres alone, abundance of fibres according to their size (micro-, meso- or macro-) and total length according to their polymer composition (Acr: acrylonitrile (acrylic); PA: polyamide; PET: polyethylene terephthalate (polyester); PE: polyethylene; PP: polypropylene) for each individual.

6.3.2. Relationship plastic load with biological variables and diet

Drivers of plastic load

No significant correlations were observed among plastic descriptors and biological variables during the detailed analysis of plastic ingestion (Fig. 4). Further analysis on the influence of size, sex and stomach fullness over the prevalence of fibres and balls and the abundance and total length of fibres also showed no significant effects ($p > 0.05$). Only for individuals sampled in August from the Clyde Sea a negative significant relationship was observed between the load of fibres and individuals' size (GLM; $z = -2.44$, $p = 0.016$) as well as a higher load of fibres in females (GLM; $z = -4.47$, $p < 0.001$), though the number of females analysed was low (5 out of 30). Analysis on the relationship with the

moulting stage was limited to the Gulf of Cadiz since most of the individuals analysed in the rest of the locations were considered to be at the intermoult stage. In the Gulf of Cadiz, a significantly reduced prevalence of fibres and mean load of fibres were seen in individuals at the post-moult stage (Fisher's exact test $p = 0.005$ for the prevalence of fibres; $z = -3.22$, $p = 0.0013$ for the abundance of fibres) compared to those at intermoult. Prevalence of balls was also marginally higher in individuals at intermoult (Fisher's exact test, $p=0.054$).

Analysis of diet composition

The diet of *Nephrops norvegicus* mainly consisted of decapod crustaceans, fish remains (great frequency of occurrence of vertebrae, otoliths and scales), gastropods and polychaetes. Unidentified items, mainly amorphous soft portions that could not be identified with certainty but in some cases resembled tissue of molluscs, also accounted for a significant proportion of diet (~20% of the total weight). Empty stomachs were frequent, particularly in the NW Iberian margin (32 out of 61), and the values of stomach fullness significantly varied among locations (KW, $X^2 = 51.63$, $df = 5$, $p < 0.001$), being the individuals from S Barcelona the ones with the lowest stomach repletion, whereas the highest values were observed in individuals from N Barcelona, the Gulf of Cadiz and Ibiza. In terms of diversity indices, significant differences were observed for Shannon's diversity index (H'; KW; $X^2 = 47.08$, $df = 6$, $p < 0.001$), with the highest values observed in the Gulf of Cadiz, but no significant differences were found in terms of evenness (J'; $p > 0.05$) (Supplementary Material Table S3).

PCoA analysis revealed great variability in the diet composition with no clear trends according to location (Fig. 5A). Results of PERMANOVA analysis showed significant differences among locations (pseudo-F=7.93, $p < 0.001$), though these results should be taken with caution since significant differences in the dispersion (variance) among groups were also observed (PERMDISP implemented in R; $p < 0.05$) and these might be driving the observed PERMANOVA differences given the unbalanced number of individuals analysed per location. Based on the relative proportion of weight for each prey group (Supplementary Material Table S3), individuals from the Gulf of Cadiz were seen to contain a high proportion of echinoderms and cnidaria representatives along with decapod crustaceans, whereas in the Mediterranean Sea, both the Balearic (except for individuals from Ibiza) and the Alboran Sea areas, were characterised by the high contribution of decapod crustaceans and unidentified items. In the continental slope of the Balearic Sea gastropods were also abundant in stomach contents. Individuals from Ibiza showed a high contribution of fish remains, polychaetes and crustaceans. Finally, in the NW Iberian margin, decapod crustaceans and fish were the main contributors followed by other crustaceans and cephalopods.

The presence of balls was associated to practically all prey items considered (Fig. 5A), with the exception of fish remains. Since the highest part of *N. norvegicus* diet is based on benthic prey (epi- and endobenthic invertebrates) the occurrence of balls seems rather associated with such compartments, particularly decapods and other crustaceans (Fig. 5B). However, no significant differences were found when comparing the diet composition of individuals with and without the presence of balls in the Gulf of Cadiz alone, neither when using the detailed diet composition (14 prey groups; unidentified items not included) nor when classifying prey items into plankton and hyperbenthos, epibenthos and endobenthos (plot not included, PERMANOVA; $p > 0.05$). In the former case, balls were – slightly - less associated with foraminiferans (small meiofauna) and euphausiids (zooplankton) (Fig. 5B). No differences were observed in terms of stomach fullness (F) or diet diversity indices (H' and J') (KW; $p > 0.05$).

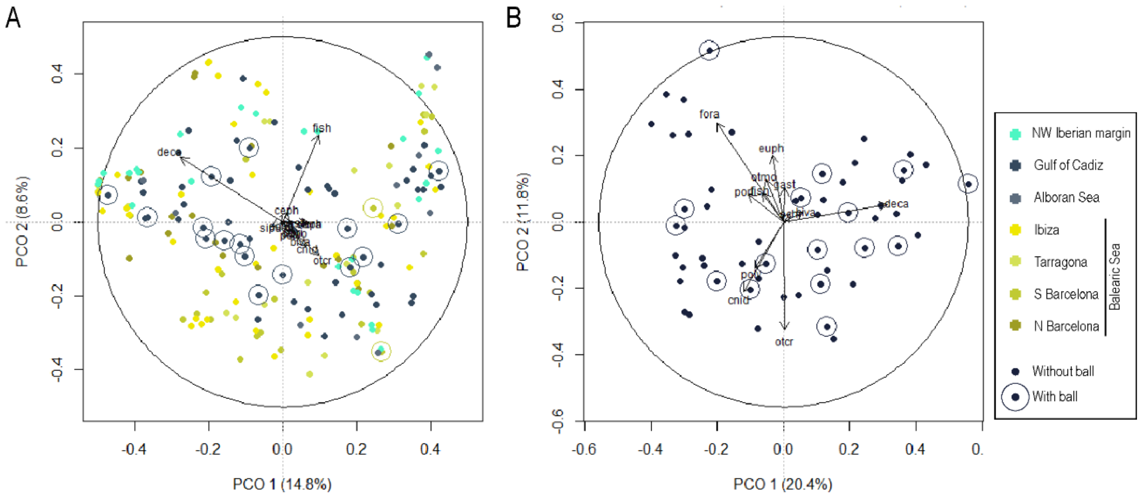


Fig. 5. Principal Coordinate Analysis (PCoA) on the diet composition (14 prey groups established) of *Nephrops norvegicus* considering (**A**) all locations sampled and (**B**) individuals of the Gulf of Cadiz alone to maximize the comparison between individuals with and without aggregations of tangled fibres (balls). Arrows depict the Spearman's correlation of each of the prey-groups considered. Acronyms used: fora – Foraminifera, pori – Porifera, sipu – Sipuncula, cnid – Cnidaria, biva – Bivalvia, ceph – Cephalopoda, gast – Gastropoda, otmo – Other molluscs, poly – Polychaeta, euph – Euphausiacea, deca – Decapoda, otcr – Other Crustacea, echi – Echinodermata, fish – Osteichthyes.

6.4 Discussion

The present study represents an extended quantification of plastic ingestion, particularly fibres, in several populations of the Norway lobster, *Nephrops norvegicus* (Linnaeus, 1978). The variability observed in the prevalence and load of fibres and balls among locations gives further support to the promising value of this species as a potential monitor species for plastic pollution. In particular, the use of the prevalence of balls alone appears to be a good affordable indicator of increased plastic ingestion when comparing among populations and time periods.

6.4.1 Description of plastic ingestion

Our results reveal that ingestion of plastics is common in this species in several locations across its geographical distribution, reaching values of up to 80-90% of prevalence of fibres and 20-30% of balls in those areas with the highest level of ingestion. Similar values of high prevalence have been repeatedly reported in the Clyde Sea (83%: Murray and Cowie, 2011;

84%: Welden and Cowie, 2016a), the North Irish Sea (83.3%: Hara et al., 2020) and in Sardinian waters (83%: Cau et al., 2019). Even a total prevalence (100%) has been identified in the Adriatic Sea (Martinelli et al., 2021), yet in this case, the characterization included particles down to 20 µm thanks to a complex process of enzymatic digestion of the digestive system followed by a high-resolution µFTIR characterization, therefore being hardly comparable to our results. In contrast, low values of prevalence in this species have also been described in other areas like the Balearic Islands (38%, Alomar et al., 2020) and the North Adriatic Sea (10%, Avio et al., 2020) and even though limited sample sizes were used (n = 8-10), in both cases these low values were attributed to potentially site-specific differences. In our case, the lowest prevalence of plastics observed, in individuals from the Ebro Delta, still represented more than 50% of the individuals with at least one plastic item. The mean abundance reported (2.15 ± 2.76) falls in the range of the maximum values identified in other locations, e.g., 2.30 ± 2.47 particles per individual in the Irish Sea and 4.65 particles per individual (estimated from abundance and prevalence values given) in the Adriatic Sea (Cau et al., 2019; Hara et al., 2020). Although none of the studies conducted in the Clyde Sea reported abundance values, the highest values of plastic load reported was > 0.2 mg (Welden and Cowie, 2016a), much higher than in the present study (we estimate that in the Gulf of Cadiz where we identified the highest load, roughly 0.038 mg of plastic were present).

In terms of size, shape and polymer composition, both similarities and differences from reported values can be found. For instance, we observed a great dominance of fibres (over 98%) as previously observed in the Clyde Sea (Murray and Cowie, 2011; Welden and Cowie, 2016a) and Irish waters (Hara et al., 2020), where both the predominance of fibres and the frequent occurrence of balls were pointed out. On the other hand, in the Adriatic Sea, Cau et al. (2019) reported that only 14% of the items observed were filaments and later Martinelli et al. (2021) also observed a greater incidence of small fragments. Neither of them reported aggregations of tangled fibres which, given their common occurrence in other locations, points out the need for checking whether their methodologies (i.e., digestion) were suitable for the identification of tangled fibres. Other similar species like the deep-sea shrimp *Aristeus antennatus* (Carreras-Colom et al., 2020, 2018) or the brown shrimp *Crangon crangon* (Devriese et al., 2015) have also been reported with a clear dominance of fibres over fragments, a bias that might reflect that fibres are more easily ingested and retained in decapod crustaceans (Welden and Cowie, 2016a). Following the identification of synthetic fibres in 67% of the specimens analysed, Avio et al. (2020) suggested that fibres might be more likely to occur along densely populated coastlines due to their potential textile origin, thus suggesting that the dominance of fibres among ingested plastics might be also related to environmental differences. Findings of great predominance or even unique presence of fibres in sediments from submarine canyons in the NW Mediterranean Sea (Sanchez-Vidal et al., 2018), sediments from the Ebro's river mouth (Simon-Sánchez et al., 2019) and coastal sediments from the Southern Portuguese shelf waters (Frias et al., 2016) provide further support to this statement of a potential predominance of fibres in environmental matrices. Among all the identified fibres, the most abundant size class observed was that of 1-2 mm, matching the observations from the Adriatic Sea (Cau et al., 2019). Thomas and Davidson (1962) investigated the size range of prey eaten and determined that the minimum food particle size that adult *N. norvegicus* were able to intentionally ingest was 1 mm and that the maximum for hard particles such as shell pieces was 5 mm. However, large-bodied organisms, such as polychaetes of up to 60 mm long could be eaten if they were ingested lengthways, which could explain why also long synthetic fibres of up to 22 mm (45 mm if fibres in aggregations of tangled fibres are also considered) were observed.

Overall, seven different synthetic polymers were identified throughout the study with PET being considered the most predominant polymer among the ingested fibres of *N. norvegicus*. This predominance has already been highlighted in other studies focused on plastic ingestion in crustaceans (Carreras-Colom et al., 2020) and matches with the fact that PET was the most abundant synthetic polymer identified in surface waters and deep-sea sediments around the world (Sanchez-Vidal et al., 2018; Suaria et al., 2020). However, these studies also identified a high proportion (>79%) of cellulosic fibres that highly contrasts with our results (cellulosic fibres contributing to roughly 2-3% of all fibres identified). In this study, two main patterns in polymer composition were observed depending on the relative importance of PET and Acr, and PA and PP fibres. For instance, a higher proportion of PET and acrylic fibres, two commonly used polymers in the textile industry, was observed in individuals sampled from the vicinity of urbanised areas (N Barcelona) where the input of textile fibres through waste-water treatment plants and stormwater overflow during episodes of increased rainfall is probably higher (Carreras-Colom et al., 2020; Siegfried et al., 2017). On the other hand, a high proportion of PA and PP as observed in the Clyde and

Gulf of Cadiz samplings might be associated with fishing effort (Cartes et al., 2016), since fishing nets are commonly made of these polymers and fishing activity in these areas is rather high. Another three synthetic polymers were identified almost exclusively in the form of fragments. Polyethylene films, like the one we identified, are one of the most common forms of marine litter in Mediterranean surface waters (Suaria et al., 2016). Less common is the observation of polychloroprene (neoprene), occasionally reported in surface waters of Barcelona (Camins et al., 2020) and sediments of the North Sea (Lorenz et al., 2019), and polydimethylsiloxane (silicone), curiously already reported in the stomach contents of Norway lobsters from the Adriatic (Cau et al., 2019).

6.4.2 Wide geographical differences and the use of balls as a proxy

All things considered, three locations were identified as having a high level of plastic ingestion, the Gulf of Cadiz, the Clyde Sea (at least in August) and the area close to Barcelona in the Balearic Sea, all of them with a prevalence of balls >20%. A feature that the three areas have in common is the potential influence of densely populated urban and industrial areas as well as the input of rivers (the Guadalquivir, the Clyde and the Llobregat, respectively) that flow through these areas and might be driving plastics of terrestrial origin towards the sampled areas as observed for other pollutants (González-Ortegón et al., 2019; Palanques et al., 2008; Vane et al., 2011). Lebreton et al. (2017) estimated that over 1.15 and 2.41 million tonnes of plastic waste are entering the oceans every year from rivers. Moreover, both the Mediterranean and the Clyde area are considered semi-enclosed bodies of water with a very low renewal rate which, in addition to the proximity of anthropogenic activities might induce a high input and lead to the accumulation of plastics with time. In fact, the Mediterranean Sea and more specifically the NW area (the Balearic Sea), have been long pointed out as potential hotspots for marine litter by several models (Coppini et al., 2018; Lebreton et al., 2012; Liubartseva et al., 2018), with great fluxes of plastic debris to the bottom expected (Coppini et al., 2018; Liubartseva et al., 2018). The Gulf of Cadiz, even though viewed as a more open area, is influenced by both the overflow of Mediterranean waters and heavy maritime traffic as the potential main sources of plastic pollution, not to mention the intense fishing activity in the area (Mecho et al., 2020) that could also explain the high abundance of PA fibres. Lost or discarded gears are frequent in the sea bottom of these and other areas of European waters, as identified in ROV video surveys (Mecho et al., 2020; Pham et al., 2014; Tubau et al., 2015).

Medium levels of plastic ingestion were identified in the Costa Brava and the Ebro Delta, where values of fibre load were half the ones reported in N Barcelona or the Gulf of Cadiz (locations with the highest levels of ingested plastics), and balls were almost absent (mostly <10%). These two locations, despite being also in the NW Mediterranean Sea, where plastic sources are allegedly high and, in the case of the Ebro Delta area, high riverine inputs of fibres are expected (Simon-Sánchez et al., 2019), may benefit from hydrodynamic currents that wash the levels of arriving plastics to the continental shelf towards deeper waters. Particularly in the Costa Brava, land-to-deep marine environment transport is favoured by the torrential regime of the Tordera river and the system of submarine canyons (Sanchez-Vidal et al., 2013) in addition to a southwards dominating current (the northern current) that has already been identified as one of the main drivers for surface plastics distribution in the area (de Haan et al., 2019). This idea is further supported again by the high prevalence of balls found in *A. antennatus* in deep waters from the same area (Carreras-Colom et al., 2020).

Lastly, and considering the observations on the prevalence of balls alone during the fast-screening analyses, two main areas were pointed out as areas of potentially lower plastic ingestion levels, the NW Iberian margin and the Alboran Sea. The incidence of meso- and macro-plastics in the stomach contents of other deep-dwelling organisms (elasmobranchs) in the area of the Bay of Biscay has also been reported as incidental (>0.03%) with a total absence of individuals with plastics in the area close to the Cabo Finisterre (López-López et al., 2017), the same area covered in this study where none of the individuals were observed with balls. Similarly, the comparison of plastic ingestion in *Scyliorhinus canicula* also suggested a lower incidence in the Galician coast compared to the NW Mediterranean or other areas surrounding the Iberian Peninsula (Bellas et al., 2016). In the Alboran Sea, the absence of balls in langoustines, assumed to be associated with reduced plastic ingestion, greatly contrasts with reported values of marine litter in the seafloor of the area (García-Rivera et al., 2018) but could be related to the great heterogeneity or the decreasing trend (2011 compared to 2007) in marine litter these authors already described in the area. The absence of other reported data on environmental concentrations or levels of plastic ingestion by marine biota in the area limits further discussion.

In conclusion, geographical differences observed through the load of plastics (abundance and total length of fibres) were considered to match the trends in the prevalence of balls, with those samplings and locations having higher mean values of ingested plastics also showing a higher prevalence of balls (>20%) whereas the absence of balls was related to low levels of ingested plastics. Therefore, the prevalence of balls was considered a good proxy to compare plastic ingestion among populations.

6.4.3 Temporal trends in the prevalence of balls

In addition to the detailed analysis of plastic ingestion in 2007 and 2018-2019, having a look at the historical records that the screening of stomach contents from 1990-1992 provide, makes it possible to evaluate to some extent the evolution of plastic ingestion in the Balearic Sea, particularly in areas close to Barcelona. Back in the 90s, the prevalence of balls was restricted to a particular area located south of the metropolitan area of Barcelona and at the mouth of one of the numerous submarine canyons that incise the continental margin. Despite not having more records in this particular area, the increase in the prevalence of balls in areas located north to the metropolitan area, going from an absolute absence to values of 35 and 20% in 2018 and 2019, suggest that plastic ingestion may have increased in the area during this period. Observations in the astonishing values of the prevalence of balls in deep-sea shrimps (over 80%) in 2018-2019 compared to values of around 10-20% in 1988-1989 give further support to a potential increase in the levels of plastic ingestion of crustaceans in the area (Carreras-Colom et al., 2020). Unfortunately, given the novelty of the study of microplastics, historical records of plastic ingestion are almost non-existent. To date, the most extensive study record is that provided by Courtene-Jones et al. (2019) that concluded that microplastic ingestion levels in the Rockall Through (North-East Atlantic) had remained relatively stable over four decades. Observations of tangled fibres in sediment cores obtained near the Columbretes Islands (ca. 60 km S of the Ebro Delta) also provide further evidence of the presence of these pollutants, at least in certain locations of the Balearic Sea, since the 1980s (radionuclide dating, *Cartes et al., unpublished*)

In the Clyde Sea area, similar trends are observed. It seems clear to some extent, given the limitations of comparing values reported through very different methodologies and giving limited details of what was observed, that plastic ingestion has increased between the 1990s and 2011, going from roughly 1% of the individuals showing some kind of inert objects that could be considered plastic (Bailey et al., 1986) to half of the individuals showing a tangle of fibres in 2011 (Murray and Cowie, 2011). Following studies suggest that plastic ingestion levels might have stayed stable or even decreased since then with values of the prevalence of balls between 18.2 and 51.7% in 2015 (Welden and Cowie, 2016a) compared to our observed values of 3.3-26.7% in 2019.

6.4.4 Relationship between ingested plastics and diet

Previous studies have described *Nephrops norvegicus* as being a non-selective species that exploits the food resources available (see references in Cristo, 1998) and dietary differences have been more related to changes in prey abundance rather than prey preference (Parslow-Williams et al., 2002). Present results match the diet composition and differences among locations reported previously (Cristo and Cartes, 1998) for S Portugal, Alboran and Balearic Sea areas. As a benthic crustacean with an opportunistic foraging behaviour and a diet rich in epi- and endobenthic fauna, the route of entry for plastic fibres in *N. norvegicus* may be hypothesised as both passive from the environment during their natural feeding or through the consumption of organisms with plastics aggregated or already ingested (trophic transfer), and active through the direct ingestion of fibres mistaken for prey items like polychaetes (Miranda and de Carvalho-Souza, 2016; Possatto et al., 2011). Direct observations during experimental exposures demonstrated that langoustines were unable to discriminate synthetic filaments from food (Murray and Cowie, 2011). Detailed analysis of diet in the deep-sea shrimp *Aristeus antennatus* revealed a positive association between the contribution of endobenthic prey and the presence of balls, suggesting a high accumulation of fibres in sediments and inter-individual differences on the levels of infauna consumption driving differences in the fibre load among shrimps (Carreras-Colom et al., 2018). We found a similar tendency, though much less significant, with the presence of balls being associated to mostly benthic prey (decapods and other crustaceans), less to fish remains. This could be explained because, in comparison to *A. antennatus*, the Norway lobster does not exploit infauna so intensively. Other factors that could be argued would be the great proportion of empty stomachs due to a presumably low rate of feeding in this species and a possibly longer period of fibre retention. In crustaceans, moulting has been proposed as the main and most plausible route of removal of plastics (Welden and Cowie, 2016b), as individuals get rid of the foregut where plastics are retained during ecdysis. Moulting in the deep-sea shrimp is thought to happen every few months (Demestre, 1995) whereas in the langoustine it may take place only once a year in mature females (Bailey et al., 1986), thus extending the period during which plastics can be ingested and accumulated and hindering the establishment of such a relationship between the presence of fibres and past meals.

6.4.5 *Nephrops norvegicus* as monitor species of plastic ingestion

The potential to retain plastic fibres poses a greater threat to organisms and adds value to the inclusion of a certain species in monitoring programs. Moreover, the potential long retention times of this species mean that it can integrate a temporal scale far beyond the last meal, in contraposition to what happens in fish where we get a snapshot of the most recent meal (Roch et al., 2021). This increased retention time may come in handy in monitoring programs, especially in the Mediterranean that features highly dynamic currents that can quickly alter environmental concentrations of plastics (de Haan et al., 2019; Kaandorp et al., 2020), but also because it may help detect low environmental values thanks to the concentration factor (considering there is a more or less constant incorporation of fibres through ingestion with time). Overall, the Norway lobster qualifies for all criteria proposed by Fossi et al. (2018) as a good indicator species. In addition to the extensive knowledge that exists on plastic ingestion in wild individuals for this species (Table 3), compared to most species studied and especially in deep-water environments, its ecology, biology and response to other stressors is well studied (i.e. feeding ecology: Cristo and Cartes, 1998; burrowing behaviour: Campbell et al., 2009; bathyal distribution: Cartes et al., 1994; stress biomarkers: Antó et al., 2009; heavy metal accumulation: Canli and Furness, 1993; organic pollutant accumulation: Perugini et al., 2007; diseases: Stentiford and Neil, 2011; trawling impact: Milligan et al., 2009). It is considered an economically and ecologically key species for which it is included in several regional and national monitoring programs (i.e. ICES and MEDITS surveys) and therefore individuals may be readily available for collection if not from established monitoring frameworks then from commercial markets.

Table 3. Review of reported data on the presence and load off plastics in stomach contents of *Nephrops norvegicus* including peer-reviewed published values, project reports and conference communications. Values in italics have been estimated from given graphics.

Sampling location	Year	n	Method	Value	Description	Reference
Clyde Sea; Little Cumbrae	ca.1986	393	Visual inspection	1.0%	prevalence of "rubber" materials [a]	(Bailey et al., 1986)
				0.2%	occurrence of "elastic" materials [a]	
Clyde Sea; Little Cumbrae	1994	42	Visual inspection	*4.7 – 15.3%*	proportion by weight of inert objects [b]	(Parslow-Williams et al., 2002)
Clyde Sea; Ailsa Craig	1994	49		*4.2 – 7.0%*	proportion by weight of inert objects [b]	
Clyde Sea; Isles of Cumbrae	2009	120	Visual inspection (400x)	83%	prevalence of plastics [a]	(Murray and Cowie, 2011)
			+ µRaman (subsample)	**50%**	**prevalence of balls**	
Clyde Sea Area	2011	1000	Visual inspection	84.1%	prevalence of microplastics [a]	(Welden and Cowie, 2016)
Main Channel	2011	658	+ FTIR (subsample)	**18.2 – 51.7%**	**prevalence of balls [a]**	
				3.3 – 3.4%	prevalence of films [a]	
				0.20 – 0.66	mg plastics	
Fairlie Channel	2011	342		**19.6 – 37.0%**	**prevalence of balls [a]**	
				3.8 – 12.7%	prevalence of films [a]	
				0.28 – 0.47	mg plastics	
North Minch	2011	150		43.0%	prevalence of microplastics [a]	
				>0.01	mg microplastics	
North Sea	2011	300		28.7%	prevalence of microplastics [a]	
				0.40	mg microplastics	
Irish coast	2016	150	Digestion	69%	prevalence of microplastics [c]	(Hara et al., 2020)
			+ (µ)FTIR (all)	1.75 ± 2.01	items · ind^{-1}	
Aran Prawn Grounds		30		56.7%	prevalence of microplastics [c]	
				0.90 ± 1.03	items · ind^{-1}	
Bantry Bay		30		73.3%	prevalence of microplastics [c]	
				1.67 ± *2.0*	items · ind^{-1}	
Kenmare Bay		30		70.0%	prevalence of microplastics [c]	
				2.30 ± 2.47	items · ind^{-1}	
Magharees Union		30		60.0%	prevalence of microplastics [c]	
				1.67 ± *1.9*	items · ind^{-1}	
North Irish Sea		30		83.3%	prevalence of microplastics [c]	
				2.20 ± *2.2*	items · ind^{-1}	

Irish coast; Galway Bay	2017	3	Digestion	0	prevalence of microplastics	(Pagter et al., 2020)
Black head			+ (µ)FTIR (subsample)			
South sound		29		0.48	items · ind^{-1}	
				1.27	items · positive ind^{-1}	
NW Mediterranean Sea; Balearic	2015	8	Visual inspection (40.5x)	38%	prevalence of microplastics [a]	(Alomar et al., 2020)
Islands				0.63 ± 0.32	microplastics · ind^{-1} [a]	
Central Mediterranean Sea;	2017	89	Density-separation	83%	prevalence of microplastics [d]	(Cau et al., 2019)
Sardinia			+ Digestion (H_2O_2)	5.5 ± 0.8	microplastics · positive ind^{-1} [e]	
			+ µFTIR (all)	117.3	mm^2 total surface of microplastics	
Central Mediterranean Sea;	-	27	Density-separation	70%	prevalence of microplastics [f]	(Cau et al., 2020)
Sardinia			+ µFTIR (all)	2.1 ± 0.6	microplastics · positive ind^{-1} [e]	
North Adriatic Sea	2016	10	Density-separation	10%	prevalence of microplastics [g]	(Avio et al., 2020)
			+ Digestion (H_2O_2)	1 ± 0	microplastics · ind^{-1}	
			+ µFTIR (all)			
Adriatic Sea	2019	23	Digestion (protease)	100%	prevalence of microplastics [h]	(Martinelli et al., 2021)
			+ FTIR (all)	4.9 ± 2.4	microplastics · ind^{-1}	

[a] *No description of size maximum, minimum or range.*

[b] *Includes synthetic fibres but also stones, spines and other indeterminate items.*

[c] *Size of particles ranged between 0.143 and 16.976 mm.*

[d] *Size of particles ranged between 0.1 and 5 mm, with some exceptions (n=4). Textile fibres excluded.*

[e] *Average value considering only positive specimens (only individuals with microplastics; intensity).*

[f] *Size of particles ranged between 0.2 and 15 mm.*

[g] *Size of particles ranged between 0.01 and 0.1 mm.*

[h] *Size of particles ranged between 51 and 286 µm for fragments and 76 and 431 µm for fibres. Items found in the intestine are also included.*

The study of plastic ingestion, which may be limited to stomach contents, may not deter the marketing of the tail, the most valuable part. Most importantly, the prevalence of balls, which may be considered as a good proxy of total plastic fibre ingestion based on our results, can be used as an easy and affordable indicator since direct visual inspection of stomach contents with low magnification is possible, thus significantly reducing the cost, time, risk of contamination and level of expertise and equipment required for sample processing. Finally, thanks to its wide geographical and bathymetric distribution it can be incorporated into global European monitoring programs for both Mediterranean and Atlantic waters. In this way, a useful indicator for meso- and macro-plastic fibres from deep (at least in the Mediterranean) environments, with the potential to integrate wide temporal scales, could be easily included in monitoring programs and complement the already settled indicators for plastic ingestion of marine biota, that is the northern fulmar and loggerhead turtle, for which the overlap in the distribution range would also allow for comparisons.

Thanks to the interest that *Nephrops norvegicus* has received in the previous decade in relation to plastic pollution, there is extensive knowledge on the levels of plastic ingestion in wild populations as well as on the potential impacts from experimental designs. Present results give further support to the idea that this species is subject to high levels of plastic ingestion (maximum levels of nearly 200 mm of fibres in a single individual) in several of its populations. Aggregations of tangled balls of fibres that also incorporate algae and other elements of its diet and may take up significant space inside the foregut are also commonly found in locations with high levels of ingested plastics. Given the complex dynamics of plastics in the sea, the biomonitoring of marine plastic debris should rely on the combination of several bioindicator species with different characteristics that complement each other (Bonanno and Orlando-Bonaca, 2018). We consider that the inclusion of *Nephrops norvegicus* as part of these monitoring programs, in particular with the evaluation of the prevalence of balls, would serve as a good affordable proxy for fibre contamination in both Mediterranean and Atlantic waters along geographical and temporal scales.

Acknowledgements

This work was partially supported by the Spanish Ministry of Science, Innovation and Universities projects “PLASMAR” (RTI2018-094806-B-100) and ECOPREST (VEM2003-20081-CO2-02), and by the Catalan Department of Agriculture, Livestock, Fisheries and Food (European Maritime and Fisheries Fund (EMFF)) project “SOMPESCA” (ARP059/19/00003). Carreras-Colom benefits from an FPU grant from the Spanish Ministry of Science, Innovation and Universities (FPU16/03430). Our thanks especially to Dr Ángel Mateo-Ramírez (IEO-Centro Oceanográfico de Málaga) and to the ISUNEPCA17 an MEDITS17 surveys for providing the material from the Gulf of Cádiz and the Alboran Sea.

References

Alomar, C., Deudero, S., Compa, M., Guijarro, B., 2020. Exploring the relation between plastic ingestion in species and its presence in seafloor bottoms. Mar. Pollut. Bull. 160, 111641. https://doi.org/10.1016/j.marpolbul.2020.111641

Antó, M., Arnau, S., Buti, E., Cortijo, V., Gutiérrez, E., Solé, M., 2009. Characterisation of integrated stress biomarkers in two deep-sea crustaceans, *Aristeus antennatus* and *Nephrops norvegicus*, from the NW fishing grounds of the Mediterranean sea. Ecotoxicol. Environ. Saf. 72, 1455–1462. https://doi.org/10.1016/j.ecoenv.2009.02.007

Avio, C.G., Pittura, L., D’Errico, G., Abel, S., Amorello, S., Marino, G., Gorbi, S., Regoli, F., 2020. Distribution and characterization of microplastic particles and textile microfibers in Adriatic food webs: General insights for biomonitoring strategies. Environ. Pollut. 258, 113766. https://doi.org/10.1016/j.envpol.2019.113766

Backhaus, T., Wagner, M., 2020. Microplastics in the Environment: Much Ado about Nothing? A Debate. Glob. Challenges 4, 1900022. https://doi.org/10.1002/gch2.201900022

Bailey, N., Howard, F.G., Chapman, C.J., 1986. Clyde *Nephrops*: biology and fisheries. Proc. R. Soc. Edinburgh. Sect. B. Biol. Sci. 90, 501–518. https://doi.org/10.1017/s0269727000005194

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026

Bonanno, G., Orlando-Bonaca, M., 2018. Perspectives on using marine species as bioindicators of plastic pollution. Mar. Pollut. Bull. 137, 209–221. https://doi.org/10.1016/j.marpolbul.2018.10.018

Camins, E., de Haan, W.P., Salvo, V.S., Canals, M., Raffard, A., Sanchez-Vidal, A., 2020. Paddle surfing for science on microplastic pollution. Sci. Total Environ. 709, 1–10. https://doi.org/10.1016/j.scitotenv.2019.136178

Campbell, N., Allan, L., Weetman, A., Dobby, H., 2009. Investigating the link between *Nephrops norvegicus* burrow density and sediment composition in Scottish waters. ICES J. Mar. Sci. 66, 2052–2059. https://doi.org/10.1093/icesjms/fsp176

Canli, M., Furness, R.W., 1993. Heavy Metals in Tissues of the Norway Lobster *Nephrops norvegicus*: Effects of Sex, Size and Season. Chem. Ecol. 8, 19–32. https://doi.org/10.1080/02757549308035297

Carpenter, E.J., Smith, K.L., 1972. Plastics on the Sargasso Sea Surface. Science (80-.). 175, 1240–1241. https://doi.org/10.1126/science.175.4027.1240

Carreras-Colom, E., Constenla, M., Soler-Membrives, A., Cartes, J.E., Baeza, M., Carrassón, M., 2020. A closer look at anthropogenic fiber ingestion in *Aristeus antennatus* in the NW Mediterranean Sea: Differences among years and locations and impact on health condition. Environ. Pollut. 263. https://doi.org/10.1016/j.envpol.2020.114567

Carreras-Colom, E., Constenla, M., Soler-Membrives, A., Cartes, J.E., Baeza, M., Padrós, F., Carrassón, M., 2018. Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*. Mar. Pollut. Bull. 133, 44–52. https://doi.org/10.1016/j.marpolbul.2018.05.012

Cartes, J.E., 1994. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea (western Mediterranean). Mar. Biol. 120, 639–648. https://doi.org/10.1007/BF00350085

Cartes, J.E., Company, J.B., Maynou, F., 1994. Deep-water decapod crustacean communities in the Northwestern Mediterranean: influence of submarine canyons and season. Mar. Biol. 120, 221–229. https://doi.org/10.1007/BF00349682

Cartes, J.E., Soler-Membrives, A., Stefanescu, C., Lombarte, A., Carrassón, M., 2016. Contributions of allochthonous inputs of food to the diets of benthopelagic fish over the northwest Mediterranean slope (to 2300 m). Deep. Res. Part I Oceanogr. Res. Pap. 109, 123–136. https://doi.org/10.1016/j.dsr.2015.11.001

Cau, A., Avio, C.G., Dessì, C., Follesa, M.C., Moccia, D., Regoli, F., Pusceddu, A., 2019. Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: Flagship species for deep-sea environments? Environ. Pollut. 255, 113107. https://doi.org/10.1016/j.envpol.2019.113107

Coppini, G., Liubartseva, S., Lecci, R., Cretì, S., Verri, G., Clementi, E., Pinardi, N., 2018. Toward 3D Modeling the Plastic Marine Debris in the Mediterranean, in: Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea. pp. 37–45. https://doi.org/10.1007/978-3-319-71279-6_6

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S.F., Narayanaswamy, B.E., 2019. Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976–2015), a study from the North East Atlantic. Environ. Pollut. https://doi.org/10.1016/j.envpol.2018.10.090

Cowger, W., Booth, A.M., Hamilton, B.M., Thaysen, C., Primpke, S., Munno, K., Lusher, A.L., Dehaut, A., Vaz, V.P., Liboiron, M., Devriese, L.I., Hermabessiere, L., Rochman, C., Athey, S.N., Lynch, J.M., De Frond, H., Gray, A., Jones, O.A.H., Brander, S., Steele, C., Moore, S., Sanchez, A., Nel, H., 2020. Reporting Guidelines to Increase the Reproducibility and Comparability of Research on Microplastics. Appl. Spectrosc. 74, 1066–1077. https://doi.org/10.1177/0003702820930292

Cristo, M., Cartes, J.E., 1998. A comparative study of the feeding ecology of *Nephrops norvegicus* L. (Decapoda: Nephropidae) in the bathyal Mediterranean and the adjacent Atlantic. Sci. Mar. 62, 81–90. https://doi.org/10.3989/scimar.1998.62s181

de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the Western Mediterranean Sea. Mar. Pollut. Bull. 140, 523–535. https://doi.org/10.1016/j.marpolbul.2019.01.053

Demestre, M., 1995. Moult activity-related spawning success in the Mediterranean deep-water shrimp *Aristeus antennatus* (Decapoda:Dendrobranchiata). Mar. Ecol. Prog. Ser. 127, 57–64. https://doi.org/10.3354/meps127057

Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., Vethaak, A.D., 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Mar. Pollut. Bull. 98, 179–187. https://doi.org/10.1016/j.marpolbul.2015.06.051

Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631–632, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046

Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019

Frias, J.P.G.L., Gago, J., Otero, V., Sobral, P., 2016. Microplastics in coastal sediments from Southern Portuguese shelf waters. Mar. Environ. Res. 114, 24–30. https://doi.org/10.1016/j.marenvres.2015.12.006

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull. 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022

García-Rivera, S., Lizaso, J.L.S., Millán, J.M.B., 2018. Spatial and temporal trends of marine litter in the Spanish Mediterranean seafloor. Mar. Pollut. Bull. 137, 252–261. https://doi.org/10.1016/j.marpolbul.2018.09.051

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment, Reports and Studies GESAMP. https://doi.org/10.13140/RG.2.1.3803.7925

González-Ortegón, E., Laiz, I., Sánchez-Quiles, D., Cobelo-Garcia, A., Tovar-Sánchez, A., 2019. Trace metal characterization and fluxes from the Guadiana, Tinto-Odiel and Guadalquivir estuaries to the Gulf of Cadiz. Sci. Total Environ. 650, 2454–2466. https://doi.org/10.1016/j.scitotenv.2018.09.290

Hara, J., Frias, J., Nash, R., 2020. Quantification of microplastic ingestion by the decapod crustacean *Nephrops norvegicus* from Irish waters. Mar. Pollut. Bull. 152, 110905. https://doi.org/10.1016/j.marpolbul.2020.110905

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297

Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of Virgin Micro- and Nanoplastics on Fish: Trends, Meta-Analysis, and Perspectives. Environ. Sci. Technol. 54, 4733–4745. https://doi.org/10.1021/acs.est.9b05995

Kaandorp, M.L.A., Dijkstra, H.A., Van Sebille, E., 2020. Closing the Mediterranean Marine Floating Plastic Mass Budget: Inverse Modeling of Sources and Sinks. Environ. Sci. Technol. 54, 11980–11989. https://doi.org/10.1021/acs.est.0c01984

Lebreton, L.C.M., Greer, S.D., Borrero, J.C., 2012. Numerical modelling of floating debris in the world's oceans. Mar. Pollut. Bull. 64, 653–661. https://doi.org/10.1016/j.marpolbul.2011.10.027

Lebreton, L.C.M., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 1–10. https://doi.org/10.1038/ncomms15611

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in mussels along the coastal waters of China. Environ. Pollut. 214, 177–184. https://doi.org/10.1016/j.envpol.2016.04.012

Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. Sci. Total Environ. 566–567, 333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084

Liubartseva, S., Coppini, G., Lecci, R., Clementi, E., 2018. Tracking plastics in the Mediterranean: 2D Lagrangian model. Mar. Pollut. Bull. 129, 151–162. https://doi.org/10.1016/j.marpolbul.2018.02.019

López-López, Lucía, Preciado, Izaskun, González-Irusta, Manuel, J., Arroyo, Isabel, Punzón, Antonio, Serrano, Alberto, 2017. Incidental ingestion of meso- and macro-plastic debris by benthic and demersal fish. Food Webs 14, 1–4. https://doi.org/10.1016/j.fooweb.2017.12.002

Lorenz, C., Roscher, L., Meyer, M.S., Hildebrandt, L., Prume, J., Löder, M.G.J., Primpke, S., Gerdts, G., 2019. Spatial distribution of microplastics in sediments and surface waters of the southern North Sea. Environ. Pollut. 252, 1719–1729. https://doi.org/10.1016/j.envpol.2019.06.093

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G

Martinelli, M., Gomiero, A., Guicciardi, S., Frapiccini, E., Strafella, P., Angelini, S., Domenichetti, F., Belardinelli, A., Colella, S., 2021. Preliminary results on the occurrence and anatomical distribution of microplastics in wild populations of *Nephrops norvegicus* from the Adriatic Sea. Environ. Pollut. 278, 116872. https://doi.org/10.1016/j.envpol.2021.116872

Mecho, A., Francescangeli, M., Ercilla, G., Fanelli, E., Estrada, F., Valencia, J., Sobrino, I., Danovaro, R., Company, J.B., Aguzzi, J., 2020. Deep-sea litter in the Gulf of Cadiz (Northeastern Atlantic, Spain). Mar. Pollut. Bull. 153, 110969. https://doi.org/10.1016/j.marpolbul.2020.110969

Menges, F., 2021. Spectragryph - optical spectroscopy software.

Miller, M.E., Kroon, F.J., Motti, C.A., 2017. Recovering microplastics from marine samples: A review of current practices. Mar. Pollut. Bull. 123, 6–18. https://doi.org/10.1016/j.marpolbul.2017.08.058

Milligan, R.J., Albalat, A., Atkinson, R.J.A., Neil, D.M., 2009. The effects of trawling on the physical condition of the Norway lobster *Nephrops norvegicus* in relation to seasonal cycles in the Clyde Sea area. ICES J. Mar. Sci. 66, 488–494. https://doi.org/10.1093/icesjms/fsp018

Miranda, D.D.A., de Carvalho-Souza, G.F., 2016. Are we eating plastic-ingesting fish? Mar. Pollut. Bull. 103, 109–114. https://doi.org/10.1016/j.marpolbul.2015.12.035

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032

Mytilineou, C., Fourtuni, A., Papacostantinou, C., 1992. Stomach content analysis of Norway lobster, *Nephrops norvegicus*, in the North Aegean Sea (Greece). Rapp Comm int Mer Medit 33, 46.

Palanques, A., Masqué, P., Puig, P., Sanchez-Cabeza, J.A., Frignani, M., Alvisi, F., 2008. Anthropogenic trace metals in the sedimentary record of the Llobregat continental shelf and adjacent Foix Submarine Canyon (northwestern Mediterranean). Mar. Geol. 248, 213–227. https://doi.org/10.1016/j.margeo.2007.11.001

Parslow-Williams, P., Goodheir, C., Atkinson, R.J.A., Taylor, A.C., 2002. Feeding energetics of the Norway lobster, *Nephrops norvegicus* in the Firth of Clyde, Scotland. Ophelia 56, 101–120. https://doi.org/10.1080/00785236.2002.10409493

Perugini, M., Visciano, P., Giammarino, A., Manera, M., Di Nardo, W., Amorena, M., 2007. Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea, Italy. Chemosphere 66, 1904–1910. https://doi.org/10.1016/j.chemosphere.2006.07.079

Pham, C.K., Ramirez-Llodra, E., Alt, C.H.S., Amaro, T., Bergmann, M., Canals, M., Company, J.B., Davies, J., Duineveld, G., Galgani, F., Howell, K.L., Huvenne, V.A.I., Isidro, E., Jones, D.O.B., Lastras, G., Morato, T., Gomes-Pereira, J.N., Purser, A., Stewart, H., Tojeira, I., Morato, T., Tubau, X., Van Rooij, D., Tyler, P.A., 2014. Marine Litter Distribution and Density in European Seas, from the Shelves to Deep Basins. PLoS One 9. https://doi.org/10.1371/journal.pone.0095839

Phuong, N.N., Poirier, L., Pham, Q.T., Lagarde, F., Zalouk-Vergnoux, A., 2018. Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life? Mar. Pollut. Bull. 129, 664–674. https://doi.org/10.1016/j.marpolbul.2017.10.054

Pielou, E.C., 1975. Ecological diversity. John Wiley & Sons, New York.

Possatto, F.E., Barletta, M., Costa, M.F., Ivar, J.A., Dantas, D. V, 2011. Plastic debris ingestion by marine catfish : An unexpected fisheries impact. Mar. Pollut. Bull. 62, 1098–1102. https://doi.org/10.1016/j.marpolbul.2011.01.036

Primpke, S., Wirth, M., Lorenz, C., Gerdts, G., 2018. Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. Anal. Bioanal. Chem. 410, 5131–5141. https://doi.org/10.1007/s00216-018-1156-x

Provencher, J.F., Ammendolia, J., Rochman, C.M., Mallory, M.L., 2019. Assessing plastic debris in aquatic food webs: What we know and don't know about uptake and trophic transfer. Environ. Rev. 27, 304–317. https://doi.org/10.1139/er-2018-0079

Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Bravo Rebolledo, E.L., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Anal. Methods Methods 9, 1454–1469. https://doi.org/10.1039/C6AY02419J

Roch, S., Ros, A.F.H., Friedrich, C., Brinker, A., 2021. Microplastic evacuation in fish is particle size-dependent. Freshw. Biol. 1–10. https://doi.org/10.1111/fwb.13687

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC - Trends Anal. Chem. 65, 47–53. https://doi.org/10.1016/j.trac.2014.10.011

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711. https://doi.org/10.1002/etc.4371

Rodríguez-Romeu, O., Constenla, M., Carrassón, M., Campoy-Quiles, M., Soler-Membrives, A., 2020. Are anthropogenic fibres a real problem for red mullets (*Mullus barbatus*) from the NW Mediterranean? Sci. Total Environ. 733, 139336. https://doi.org/10.1016/j.scitotenv.2020.139336

Sanchez-Vidal, A., Higueras, M., Martí, E., Liquete, C., Calafat, A., Kerhervé, P., Canals, M., 2013. Riverine transport of terrestrial organic matter to the North Catalan margin, NW Mediterranean Sea. Prog. Oceanogr. 118, 71–80. https://doi.org/10.1016/j.pocean.2013.07.020

Sanchez-Vidal, A., Thompson, R.C., Canals, M., de Haan, W.P., 2018. The imprint of microfibres in southern European deep seas. PLoS One 13, e0207033. https://doi.org/10.1371/journal.pone.0207033

Shannon, C.E., Weaver, W., 1948. The mathematical theory of communication. MD Comput. Comput. Med. Pract. https://doi.org/10.1145/584091.584093

Siegfried, M., Koelmans, A.A., Besseling, E., Kroeze, C., 2017. Export of microplastics from land to sea. A modelling approach. Water Res. 127, 249–257. https://doi.org/10.1016/j.watres.2017.10.011

Simon-Sánchez, L., Grelaud, M., Garcia-Orellana, J., Ziveri, P., 2019. River Deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean). Sci. Total Environ. 687, 1186–1196. https://doi.org/10.1016/j.scitotenv.2019.06.168

Stentiford, G.D., Neil, D.M., 2011. Diseases of *Nephrops* and *Metanephrops*: A review. J. Invertebr. Pathol. 106, 92–109. https://doi.org/10.1016/j.jip.2010.09.017

Suaria, G., Achtypi, A., Perold, V., Lee, J.R., Pierucci, A., Bornman, T.G., Aliani, S., Ryan, P.G., 2020. Microfibers in oceanic surface waters: A global characterization. Sci. Adv. 6, eaay8493. https://doi.org/10.1126/sciadv.aay8493

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 37551. https://doi.org/10.1038/srep37551

Swynnerton, G.H., Worthington, E.B., 1940. Note on the Food of Fish in Haweswater (Westmorland). J. Anim. Ecol. 9, 183–187.

Thomas, H.J., Davidson, C., 1962. The food of the Norway lobster *Nephrops norvegicus* (L.). HM Station. Off.

Torre, M., Digka, N., Anastasopoulou, A., Tsangaris, C., Mytilineou, C., 2016. Anthropogenic microfibres pollution in marine biota. A new and simple methodology to minimize airborne contamination. Mar. Pollut. Bull. 113, 55–61. https://doi.org/10.1016/j.marpolbul.2016.07.050

Tubau, X., Canals, M., Lastras, G., Rayo, X., Rivera, J., Amblas, D., 2015. Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: The role of hydrodynamic processes. Prog. Oceanogr. 134, 379–403. https://doi.org/10.1016/j.pocean.2015.03.013

UNEP/MAP SPA/RAC, 2018. Defining the most representative species for IMAP Candidate Indicator 24. By Fr. Galgani. SPA/RAC, Tunis.

Vane, C.H., Chenery, S.R., Harrison, I., Kim, A.W., Moss-Hayes, V., Jones, D.G., 2011. Chemical signatures of the Anthropocene in the Clyde estuary, UK: sediment-hosted Pb, 207/206 Pb, total petroleum hydrocarbon, polyaromatic hydrocarbon and polychlorinated biphenyl pollution records. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 369, 1085–1111. https://doi.org/10.1098/rsta.2010.0298

Welden, N.A.C., Cowie, P.R., 2016a. Environment and gut morphology influence microplastic retention in langoustine, *Nephrops norvegicus*. Environ. Pollut. 214, 859–865. https://doi.org/10.1016/j.envpol.2016.03.067

Welden, N.A.C., Cowie, P.R., 2016b. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ. Pollut. 218, 895–900. https://doi.org/10.1016/j.envpol.2016.08.020

Zhu, X., Nguyen, B., You, J.B., Karakolis, E., Sinton, D., Rochman, C., 2019. Identification of Microfibers in the Environment Using Multiple Lines of Evidence. Environ. Sci. Technol. 53, 11877–11887. https://doi.org/10.1021/acs.est.9b05262

SUPPLEMENTARY MATERIAL

Table S1. Categorization of fibres observed in *Nephrops norvegicus* based on visual appearance with optical microscopy (400x) and results on the ATR-FTIR identification. Polymers identified: PET = Polyethylene terephthalate (polyester); PA = Polyamide; Acr = Acrylonitrile (acrylic); PP = polypropylene; PE = polyethylene; Cel = Cellulose.

Cat.	Description	Polymer identified (%)					
		PET	PA	Acr	PP	PE	Cel.
A	• Mostly uniform diameter (sometimes with molten bends) and round cross-section • Clean ends, sometimes molten • Smooth surface texture. Refringent. Usually with delustrant agents visible as a bubbly backbone texture • Generally transparent or bright coloured	**85.2**	9.3	3.7	1.9	0	0
B	• Uniform diameter and round cross-section • Sometimes with wide molten or frayed ends • Generally smooth surface texture. Granular backbone texture. Pilling or fraying surface when damaged • Mostly transparent, yellowed or brownish	20.0	**76.0**	0	4.0	0	0
C	• Non-uniform diameter with dumbbell cross-section • Usually with fraying ends • Smooth and homogeneous surface and backbone texture • Mostly transparent or bright colours	11.1	5.6	**83.3**	0	0	0
D	• Mostly uniform diameter and round cross-section • Usually with fraying ends • Multiple cracks and surface marks. Sometimes fraying surface • Generally yellowed or brownish, rarely with bright colours	0	14.3	0	**85.7**	0	0
E	• Non-uniform diameter with appearance of trilobal cross-section • Clean ends, molten to some extent • Multiple cracks and surface marks. Highly refringent • Mostly transparent	0	0	0	0	**100**	0
F	• Non-uniform diameter, flat or film-like • Diagonal-cut ends • Striated surface with angular edges. Sometimes fraying surface • Mostly transparent, blue or black, usually non-uniform	0	0	0	0	0	**100**

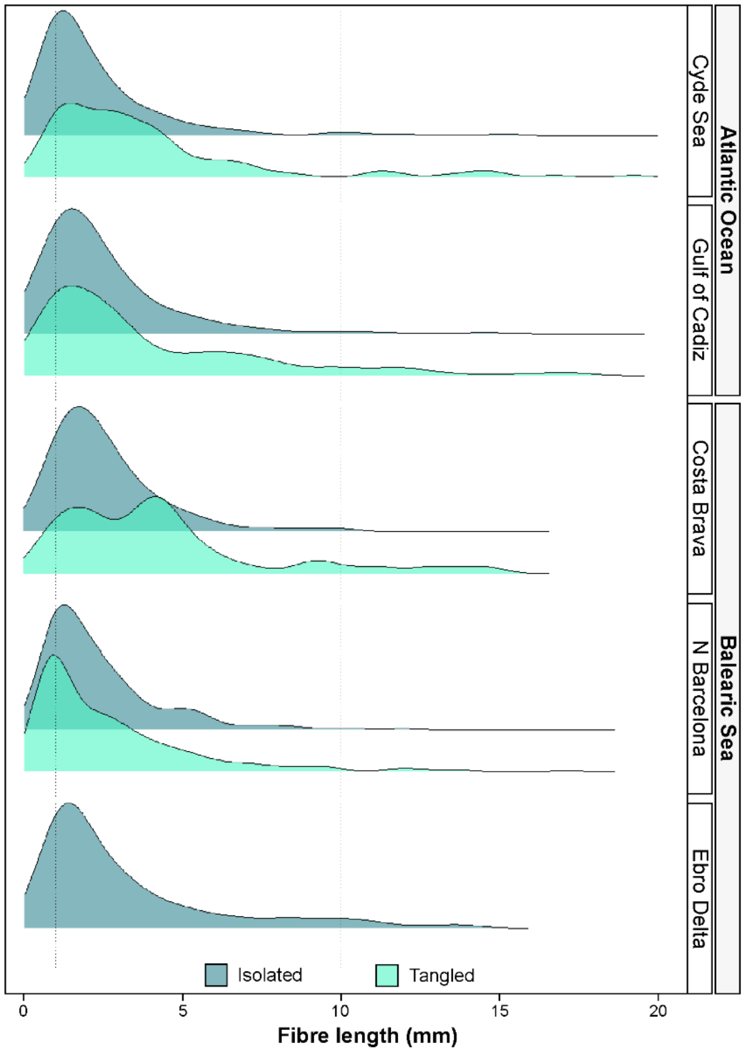


Fig. S1. Density curves of the distribution of sizes of isolated and tangled fibres according to the sampling location of the individuals (area under the curve = 1). Six fibres with lengths > 20 mm (one from N Barcelona, one from the Costa Brava, and four from the Clyde Sea individuals) excluded to improve visualization.

Table S2. Mean values (± SD) of abundance (number of fibres, n · ind^{-1}) and total fibre length (mm·ind^{-1}) of synthetic fibres in stomach contents of *Nephrops norvegicus* sampled from the Clyde Sea (CS; two samplings included), the Gulf of Cadiz (GC) and several sites along the Balearic Sea (Costa Brava, CB, N Barcelona, NB, and Ebro Delta, ED) according to their polymer composition (Acr: acrylonitrile (acrylic); PA: polyamide; PE: polyethylene; PET: polyethylene terephthalate (polyester); PP: polypropylene). Mean values of relative contribution to the total load of synthetic fibres in percentage is noted in parenthesis. For details on samplings performed see Table 1.

Sampling	Acr.		PA		PE		PET		PP	
Abundance (n · ind^{-1})										
CS1	0.57 ± 2.75	*(8.5)*	1.07 ± 1.80	*(58.7)*	0.00 ± 0.00	*(0)*	0.40 ± 1.07	*(17.9)*	0.73 ± 3.46	*(14.9)*
CS2	0.27 ± 0.91	*(2.1)*	4.47 ± 7.29	*(36.3)*	0.00 ± 0.00	*(0)*	5.50 ± 7.66	*(50.7)*	1.00 ± 2.35	*(6.9)*
GC	0.46 ± 0.88	*(9.3)*	3.75 ± 4.13	*(34.7)*	0.00 ± 0.00	*(0)*	8.21 ± 11.36	*(51)*	0.67 ± 1.99	*(4.4)*
CB1	0.35 ± 0.99	*(15.6)*	0.90 ± 1.17	*(46.5)*	0.00 ± 0.00	*(0)*	1.15 ± 1.87	*(31.3)*	0.10 ± 0.45	*(6.7)*
CB2	1.15 ± 2.66	*(16.2)*	1.20 ± 1.28	*(29.2)*	0.00 ± 0.00	*(0)*	3.55 ± 5.03	*(45.2)*	0.30 ± 0.80	*(5.8)*
NB0	1.65 ± 3.47	*(16.3)*	1.15 ± 1.46	*(20.8)*	0.00 ± 0.00	*(0)*	6.45 ± 10.16	*(60.7)*	0.15 ± 0.49	*(1.5)*
NB1	1.40 ± 2.23	*(19.4)*	1.50 ± 3.17	*(11.5)*	0.00 ± 0.00	*(0)*	7.40 ± 9.91	*(62.7)*	0.10 ± 0.31	*(1.3)*
NB2	4.30 ± 15.49	*(20.4)*	1.70 ± 2.20	*(28.3)*	0.05 ± 0.22	*(0.3)*	6.40 ± 14.30	*(49.6)*	0.10 ± 0.45	*(1)*
ED1	0.15 ± 0.49	*(8.3)*	0.65 ± 1.66	*(19.6)*	0.05 ± 0.22	*(0.9)*	1.25 ± 2.36	*(61.6)*	0.05 ± 0.22	*(2.4)*
ED2	1.65 ± 2.13	*(34.5)*	0.90 ± 1.83	*(13.2)*	0.00 ± 0.00	*(0)*	2.15 ± 2.06	*(44.1)*	0.50 ± 1.40	*(5.5)*
Total fibre length (mm · ind^{-1})										
CS1	0.41 ± 1.80	*(7.9)*	3.19 ± 6.72	*(59.4)*	0.00 ± 0.00	*(0)*	0.55 ± 1.36	*(17.5)*	2.26 ± 11.18	*(14.4)*
CS2	0.87 ± 2.81	*(2)*	11.61 ± 16.29	*(36)*	0.00 ± 0.00	*(0)*	20.22 ± 29.65	*(49.4)*	3.76 ± 8.08	*(10)*
GC	2.00 ± 3.35	*(12.6)*	11.16 ± 12.74	*(33.7)*	0.00 ± 0.00	*(0)*	25.55 ± 43.07	*(47.1)*	2.08 ± 5.57	*(5.7)*
CB1	0.93 ± 2.71	*(14.8)*	3.37 ± 7.35	*(51.1)*	0.00 ± 0.00	*(0)*	2.36 ± 4.23	*(27.4)*	0.26 ± 1.17	*(6.7)*
CB2	2.42 ± 5.35	*(12.3)*	3.18 ± 3.62	*(27.8)*	0.00 ± 0.00	*(0)*	13.39 ± 18.25	*(51)*	0.47 ± 1.26	*(3.5)*
NB0	3.93 ± 7.99	*(15.4)*	1.88 ± 2.57	*(19.5)*	0.00 ± 0.00	*(0)*	14.82 ± 20.83	*(61.3)*	0.92 ± 3.01	*(3.1)*
NB1	4.10 ± 8.88	*(18.5)*	3.89 ± 7.13	*(10.8)*	0.00 ± 0.00	*(0)*	25.08 ± 34.54	*(59.6)*	1.09 ± 3.45	*(3.4)*
NB2	4.81 ± 12.25	*(21.4)*	4.75 ± 5.48	*(29.4)*	0.22 ± 0.99	*(0.6)*	18.08 ± 40.21	*(46.8)*	0.60 ± 2.67	*(1.8)*
ED1	0.34 ± 1.18	*(9.1)*	0.93 ± 2.44	*(17)*	0.06 ± 0.27	*(0.4)*	5.61 ± 9.91	*(67)*	0.17 ± 0.74	*(1.8)*
ED2	4.97 ± 5.69	*(38.6)*	1.91 ± 3.67	*(9.1)*	0.00 ± 0.00	*(0)*	8.09 ± 9.47	*(44.8)*	1.76 ± 5.56	*(6.2)*

Table S3. Data on diet composition of *Nephrops norvegicus* including mean values of stomach fullness (F), diversity (Shannon-Wiener diversity index, H') and evenness (Pielou's index of evenness, J') and the contribution of 15 prey-groups in terms of frequency of occurrence (%F) and total weight per 100 individuals (W_{100}). *n* indicates the total number of individuals screened and the number of empty stomachs (not included in diet composition analysis) in parenthesis. * indicates a value <0.1%. n.d. indicates absence of data (total weight of individuals was not recorded). Different superscript letters among rows indicate significant differences among locations.

	NW Iberian margin		Gulf of Cadiz		Alboran Sea		Balearic Sea							
							N Barcelona		S Barcelona		Tarragona		Ibiza	
n	61 (32)		56 (1)		10 (0)		31 (6)		51 (26)		21 (3)		64 (23)	
F	1.20 ± 1.06 [a]		1.54 ± 0.72 [a]		1.15 ± 0.43 [a]		2.29 ± 3.27 [a]		0.28 ± 0.32 [b]		n.d.		1.62 ± 1.00 [a]	
H'	0.42 ± 0.38 [a]		0.96 ± 0.41 [b]		0.49 ± 0.36 [a]		0.41 ± 0.42 [a]		0.49 ± 0.38 [a]		0.67 ± 0.43 [a]		0.51 ± 0.34 [a]	
J'	0.59 ± 0.26 [a]		0.58 ± 0.22 [a]		0.40 ± 0.30 [a]		0.66 ± 0.23 [a]		0.69 ± 0.18 [a]		0.61 ± 0.24 [a]		0.61 ± 0.21 [a]	
	%F	W_{100}	*%F*	W_{100}	*%F*	W_{100}	*%F*	W_{100}	*%F*	W_{100}	*%F*	W_{100}	*%F*	W_{100}
Foraminifera	10.3	0.2	65.5	1.3	50	0.1	-	-	5.1	0.8	5	*	5.8	*
Porifera	-	-	41.8	1.3	-	-	-	-	-	-	-	-	7.7	0.7
Sipuncula	-	-	-	-	-	-	14.8	1.2	-	-	5	0.2	11.5	0.2
Cnidaria	-	-	72.7	2.3	10	0.2	-	-	7.7	0.2	10	0.1	7.7	0.3
Bivalvia	3.4	0.5	18.2	0.1	-	-	-	-	2.6	*	30	0.3	3.8	0.2
Cephalopoda	20.7	4.1	-	-	-	-	11.1	0.3	2.6	*	15	0.2	7.7	0.3
Gastropoda	20.7	0.8	40	0.2	-	-	33.3	10	23.1	0.4	20	0.4	7.7	0.3
Other Mollusca	-	-	5.5	0.2	20	0.8	11.1	0.3	2.6	0.1	10	*	13.5	0.5
Polychaeta	13.8	0.2	50.9	2.2	40	3.2	11.1	0.7	5.1	0.5	5	0.1	36.5	1.8
Euphausiacea	-	-	10.9	0.2	20	0.5	-	-	10.3	0.2	35	0.8	1.9	*
Decapoda	48.3	11.9	72.7	8.6	40	8	51.9	6.8	51.3	0.9	65	1.9	25	3.7
Other Crustacea	34.5	5	40	1.7	50	2.2	7.4	0.4	15.4	0.2	15	0.4	21.2	0.4
Echinodermata	6.9	0.5	61.8	3	30	0.4	7.4	0.1	10.3	*	25	*	5.8	1
Osteichthyes (fish remains)	44.8	5.4	54.5	2.1	50	2.1	22.2	0.8	25.6	0.2	45	1.2	42.3	2
Unidentified material	24.1	2.7	1.8	*	40	21.1	44.4	11.9	43.6	1	40	1.1	13.5	0.6

CHAPTER 7 Conclusions

7 Conclusions

7.1 Plastic ingestion is common throughout the Balearic Sea in the two bathyal decapod crustaceans analysed, *Aristeus antennatus* and *Nephrops norvegicus*, reaching depths of 1870 m in the former. They both exhibit varied but overall high values of plastic ingestion compared to those reported for other marine organisms in the area (i.e., fish). (Ch. III-VI)

7.2 Plastics ingested by both species, *A. antennatus* and *N. norvegicus*, are vastly dominated by synthetic fibres of lengths that fall mostly in the mesoplastics size range (following Hartmann et al. (2019) size classification for plastics) or the upper size range considered for microplastics (1-5 mm) (following Frias and Nash (2019)). Polyethylene terephthalate (~40-50%), acrylonitrile (~12-40%) and polyamide (9-30%) were the dominant polymers in both species. (Ch. III-VI)

7.3 Based on pieces of evidence of the presence of fragments and films in bottom water and sediment samples from the exact same locations where *N. norvegicus* were sampled, the predominance of fibres in stomach contents might be the result of either an active feeding on fibres or the fact that they are more easily retained and thus the likelihood of encountering them is increased. Even though research focusing on the plastics present in bottom water and sediments at greater depths (~800 m) would be needed to confirm it, the predominance of fibres in *A. antennatus* could have a similar explanation. (Ch. V)

7.4 Considering the high values of plastics found in the stomach compared to intestine contents in both species, in addition to the presence and overall size of tangled up fibres (balls) compared to the sizes of the pyloric chamber and the entrance to the midgut (first section of the intestine), the expulsion of plastics through faeces is considered a minor path for the removal of microplastics. Further research would be needed to assess the potential fragmentation into nanoplastics and their fate. (Ch. IV-V)

7.5 Detailed analysis on diet composition in *A. antennatus* revealed that individuals with plastics also showed higher relative biomass and diversity of endobenthic prey (e.g., decapod *Calocaris macandreae*, polychaetes, bivalves) as well as a lower relative diversity of hyperbenthic prey (e.g., mysids, decapod *Plesionika martia*). This association suggests that shrimps preying on the surface of sediment might be more exposed to the uptake of plastics either through trophic transfer or passive ingestion while feeding. (Ch. III)

7.6 Based on the similarities in size distribution and patterns in abundance among sites observed for environmental and ingested plastics, water seems to have a more important role in the exposure and uptake of plastics by *N. norvegicus*. However, analysis of diet composition in relation to the presence of plastics did not provide further support to this idea since a great proportion of benthic prey was always present. (Ch V-VI)

7.7 Observations on plastic ingestion in *A. antennatus* sampled close to Barcelona in different time periods do not suggest an increasing nor a decreasing trend in total plastic ingestion. A significant shift in the polymer composition (2007-2008 vs 2017-2018) was observed with a higher contribution of polyester fibres in individuals from 2017-2018 compared to those from 2007-2008 with a higher contribution of acrylonitrile instead. These differences are considered a reflection of the global production and usage trends in plastic fibres. (Ch. III-IV)

7.8 Individuals of *A. antennatus* sampled in 2018 showed a high spatial and seasonal variability in the levels of plastic ingestion: greater fibre loads towards the south were observed in spring, while in summer, individuals sampled off Barcelona showed a fibre load nearly thirty times higher than those from other locations. These trends might be related to the hydrodynamics of the area (e.g., a southwards current that would lead to a progressive accumulation of plastics along its path) and the increased arrival of plastics due to elevated river discharge after storm-like events. (Ch. IV)

7.9 Higher abundances and loads (sum of fibre lengths or particles areas) were observed in individuals of *N. norvegicus* and environmental samples off Barcelona compared to other areas sampled along the Catalan coast. This reinforces the hypothesis that: (1) the metropolitan area of Barcelona might be a significant source of plastic pollution, and (2) that the levels of plastic ingestion are highly correlated with environmental levels of plastic pollution. (Ch. V)

7.10 Varied levels of plastic pollution throughout European waters were identified by using the prevalence of aggregations of tangled fibres in *N. norvegicus* as an indicator of plastic pollution. Areas with potentially high values of plastic pollution were the Balearic Sea (Barcelona), the Gulf of Cadiz and the Clyde Sea, whereas the Alboran Sea and the NW Iberian margin were considered areas of low plastic pollution. (Ch. VI)

7.11 Concentrations of heavy metals in abdominal muscle did not display a clear site-related pattern and were considered in the range of previously reported tissue levels. Cd and Pb did not exceed the threshold levels determined by the European Food Safety Agency for shellfish. Sediment levels of most metal species analysed displayed higher levels in Barcelona, and levels of Ni and As in all locations sampled were above the thresholds established as probable effects concentrations. Even though natural sources might be playing a key role in the increased values of As, further research would be needed to properly assess its potential environmental effects. (Ch. V)

7.12 No histological alterations potentially related to plastic ingestion or any other potential stressor were observed in target organs of *A. antennatus.* and *N. norvegicus* (Ch. IV-V)

7.13 Despite posing a major threat for their potentially increased retention, the presence of tangled balls of fibres was not correlated with a significant impact on health condition in *A. antennatus* or *N. norvegicus.* (Ch. IV-V)

7.14 Negative correlations between plastic ingestion in *A. antennatus* and condition indices were not observed in most individuals, except for those from Barcelona, sampled in summer 2018 for which a negative correlation between GSI and fibre load was observed. Given the positive correlation between plastics and stomach fullness, it is considered unlikely that high values of ingested plastics were responsible for reduced values of GSI due to a reduction in the feeding activity and, therefore, of the nutritional state. The potential effect of the exposure to other pollutants with similar sources and dispersion pathways has been suggested instead (Ch. III-IV).

7.15 No correlations have been established between the variability in condition indices and levels of pollutants in *N. norvegicus*. High levels of HSI in the Ebro Delta could be related to both favourable environmental conditions as well as a chronic toxic response, though the former is rendered more likely based on histological observations of the hepatopancreas. (Ch. V).

7.16 The inhibition of CAT and increased LDH activities in individuals of *N. norvegicus* with high levels of ingested plastics match the enzymatic responses identified in experimental exposures to plastics and suggests a potential oxidative stress response. (Ch. V)

7.17 The prevalence of aggregations of tangled up fibres in *N. norvegicus* is significantly correlated with total abundance of ingested plastics and given its methodological advantages (i.e., fast, needs a small number of resources and training, and is less subject to overestimation because of contamination of the samples) it is proposed as an affordable indicator for plastic pollution that could be easily implemented in existing monitoring frameworks. (Ch. VI)

References

Alomar, C., Deudero, S., Compa, M., Guijarro, B., 2020. Exploring the relation between plastic ingestion in species and its presence in seafloor bottoms. Mar. Pollut. Bull. 160, 111641. https://doi.org/10.1016/j.marpolbul.2020.111641

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep. Res. Part I Oceanogr. Res. Pap. 74, 11–13. https://doi.org/10.1016/j.dsr.2012.12.008

Andrady, A.L., Neal, M.A., 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc. B Biol. Sci. 364, 1977–1984. https://doi.org/10.1098/rstb.2008.0304

Arthur, C., Baker, J., Bamford, H., 2009. Proceedings of the International Research Workshop on the Occurrence , Effects , and Fate of Microplastic Marine Debris, Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. Sept 9-11, 2008.

Avio, C.G., Pittura, L., D'Errico, G., Abel, S., Amorello, S., Marino, G., Gorbi, S., Regoli, F., 2020. Distribution and characterization of microplastic particles and textile microfibers in Adriatic food webs: General insights for biomonitoring strategies. Environ. Pollut. 258, 113766. https://doi.org/10.1016/j.envpol.2019.113766

Backhaus, T., Wagner, M., 2020. Microplastics in the Environment: Much Ado about Nothing? A Debate. Glob. Challenges 4, 1900022. https://doi.org/10.1002/gch2.201900022

Barange, M., Field, J.G., Harris, R., Hofmann, E.E., Perry, R.I., Werner, F., 2011. Marine Ecosystems and Global. Oxford University Press, New York.

Battisti, C., Gippoliti, S., 2019. Not just trash! Anthropogenic marine litter as a 'charismatic threat'' driving citizen-based conservation management actions.' Anim. Conserv. 22, 311–313. https://doi.org/10.1111/acv.12473

Baulch, S., Perry, C., 2014. Evaluating the impacts of marine debris on cetaceans. Mar. Pollut. Bull. 80, 210–221. https://doi.org/10.1016/j.marpolbul.2013.12.050

Bergmann, M., Gutow, L., Klages, M., 2015. Marine anthropogenic litter, Marine Anthropogenic Litter. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-16510-3

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B., Gerdts, G., 2017. High quantities of microplastic in Arctic deep-sea sediments from the HAUSGARTEN observatory. Environ. Sci. Technol. acs.est.7b03331. https://doi.org/10.1021/acs.est.7b03331

Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. Front. Ecol. Environ. 15, 84–90. https://doi.org/10.1002/fee.1450

Bijker, W.E., 1987. The Social Construction of Bakelite: Toward a Theory of Invention, in: The Social Construction of Technological Systems: New Directions in the Sociology and History of Technology. MIT Press, pp. 159–187.

Bonanno, G., Orlando-Bonaca, M., 2018. Perspectives on using marine species as bioindicators of plastic pollution. Mar. Pollut. Bull. 137, 209–221. https://doi.org/10.1016/j.marpolbul.2018.10.018

Bordbar, L., Kapiris, K., Kalogirou, S., Anastasopoulou, A., 2018. First evidence of ingested plastics by a high commercial shrimp species (*Plesionika narval*) in the eastern Mediterranean. Mar. Pollut. Bull. 136, 472–476. https://doi.org/10.1016/j.marpolbul.2018.09.030

Bour, A., Avio, C.G., Gorbi, S., Regoli, F., Hylland, K., 2018a. Presence of microplastics in benthic and epibenthic organisms: Influence of habitat, feeding mode and trophic level. Environ. Pollut. 243, 1217–1225. https://doi.org/10.1016/j.envpol.2018.09.115

Bour, A., Haarr, A., Keiter, S., Hylland, K., 2018b. Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environ. Pollut. 236, 652–660. https://doi.org/10.1016/j.envpol.2018.02.006

Brandts, I., Teles, M., Gonçalves, A.P., Barreto, A., Franco-Martinez, L., Tvarijonaviciute, A., Martins, M.A., Soares, A.M.V.M., Tort, L., Oliveira, M., 2018a. Effects of nanoplastics on *Mytilus galloprovincialis* after individual and combined exposure with carbamazepine. Sci. Total Environ. 643, 775–784. https://doi.org/10.1016/j.scitotenv.2018.06.257

Brandts, I., Teles, M., Tvarijonaviciute, A., Pereira, M.L., Martins, M.A., Tort, L., Oliveira, M., 2018b. Effects of polymethylmethacrylate nanoplastics on *Dicentrarchus labrax*. Genomics 110, 435–441. https://doi.org/10.1016/j.ygeno.2018.10.006

Brown, J., Macfadyen, G., 2007. Ghost fishing in European waters: Impacts and management responses. Mar. Policy 31, 488–504. https://doi.org/10.1016/j.marpol.2006.10.007

Browne, M.A., Galloway, T., Thompson, R., 2007. Microplastic - An emerging contaminant of potential concern? Integr. Environ. Assess. Manag. 3, 559–566.

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23, 2388–2392. https://doi.org/10.1016/j.cub.2013.10.012

Buchanan, J.B., 1971. Pollution by synthetic fibres. Mar. Pollut. Bull. 2, 23.

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37, 2776–2796. https://doi.org/10.1002/etc.4268

Camins, E., de Haan, W.P., Salvo, V.S., Canals, M., Raffard, A., Sanchez-Vidal, A., 2020. Paddle surfing for science on microplastic pollution. Sci. Total Environ. 709, 1–10. https://doi.org/10.1016/j.scitotenv.2019.136178

Carbonell, a., Dos Santos, A., Alemany, F., Vélez-Belchi, P., 2010. Larvae of the red shrimp

Aristeus antennatus (Decapoda: Dendrobranchiata: Aristeidae) in the Balearic Sea: new occurrences fifty years later. Mar. Biodivers. Rec. 3, e103. https://doi.org/10.1017/S1755267210000758

Carbonell, A., Carbonell, M., Demestre, M., Grau, A., Monserrat, S., 1999. The red shrimp Aristeus antennatus (Risso, 1816) fishery and biology in the Balearic Islands, Western Mediterranean. Fish. Res. 44, 1–13. https://doi.org/10.1016/S0165-7836(99)00079-X

Carbonell, A., Lloret, J., Demestre, M., 2008. Relationship between condition and recruitment success of red shrimp (*Aristeus antennatus*) in the Balearic Sea (Northwestern Mediterranean). J. Mar. Syst. 71, 403–412. https://doi.org/10.1016/j.jmarsys.2007.02.028

Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene Spherules in Coastal Waters. Science (80-.). 178, 749–750.

Carpenter, E.J., Smith, K.L., 1972. Plastics on the Sargasso Sea Surface. Science (80-.). 175, 1240–1241. https://doi.org/10.1126/science.175.4027.1240

Cartes, J.E., 1994. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea (western Mediterranean). Mar. Biol. 120, 639–648. https://doi.org/10.1007/BF00350085

Cartes, J.E., Company, J.B., Maynou, F., 1994. Deep-water decapod crustacean communities in the Northwestern Mediterranean: influence of submarine canyons and season. Mar. Biol. 120, 221–229. https://doi.org/10.1007/BF00349682

Cartes, J.E., López-Pérez, C., Carbonell, A., 2017. Condition and recruitment of *Aristeus antennatus* at great depths (to 2,300 m) in the Mediterranean: Relationship with environmental factors. Fish. Oceanogr. 27, 114–126. https://doi.org/10.1111/fog.12237

Cartes, J.E., Papiol, V., Guijarro, B., 2008. The feeding and diet of the deep-sea shrimp *Aristeus antennatus* off the Balearic Islands (Western Mediterranean): Influence of environmental factors and relationship with the biological cycle. Prog. Oceanogr. 79, 37–54. https://doi.org/10.1016/j.pocean.2008.07.003

Cartes, J.E., Sardà, F., 1993. Zonation of deep-sea decapod fauna in the Catalan Sea (Western Mediterranean). Mar. Ecol. Prog. Ser. 94, 27–34. https://doi.org/10.3354/meps094027

Cartes, J.E., Sardà, F., 1989. Feeding ecology of the deep-water aristeid crustacean *Aristeus antennatus*. Mar. Ecol. Prog. Ser. 54, 229–238. https://doi.org/10.3354/meps054229

Cau, A., Avio, C.G., Dessì, C., Follesa, M.C., Moccia, D., Regoli, F., Pusceddu, A., 2019. Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: Flagship species for deep-sea environments? Environ. Pollut. 255, 113107. https://doi.org/10.1016/j.envpol.2019.113107

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2011.09.025

Colin, N., Porte, C., Fernandes, D., Barata, C., Padrós, F., Carrassón, M., Monroy, M., Cano-Rocabayera, O., de Sostoa, A., Piña, B., Maceda-Veiga, A., 2016. Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers. Sci. Total Environ. 540, 307–323. https://doi.org/10.1016/j.scitotenv.2015.06.099

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F.B.R., Aguzzi, J., Ballesteros, E., Bianchi, C.N., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Froglia, C., Galil, B.S., Gasol, J.M., Gertwage, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., Kitsos, M.S., Koukouras, A., Lampadariou, N., Laxamana, E., de la Cuadra, C.M.L.F., Lotze, H.K., Martin, D., Mouillot, D., Oro, D., Raicevich, S., Rius-Barile, J., Saiz-Salinas, J.I., Vicente, C.S., Somot, S., Templado, J., Turon, X., Vafidis, D., Villanueva, R., Voultsiadou, E., 2010. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. PLoS One 5. https://doi.org/10.1371/journal.pone.0011842

Colmenero, A.I., Barría, C., Broglio, E., García-Barcelona, S., 2017. Plastic debris straps on threatened blue shark *Prionace glauca*. Mar. Pollut. Bull. 115, 436–438. https://doi.org/10.1016/j.marpolbul.2017.01.011

Colton, J.B., Knapp, F.D., Bums, B.R., 1974. Plastic Particles in Surface. Science (80-.). 185, 491–497.

Coppini, G., Liubartseva, S., Lecci, R., Cretì, S., Verri, G., Clementi, E., Pinardi, N., 2018. Toward 3D Modeling the Plastic Marine Debris in the Mediterranean, in: Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea. pp. 37–45. https://doi.org/10.1007/978-3-319-71279-6_6

Cowger, W., Booth, A.M., Hamilton, B.M., Thaysen, C., Primpke, S., Munno, K., Lusher, A.L., Dehaut, A., Vaz, V.P., Liboiron, M., Devriese, L.I., Hermabessiere, L., Rochman, C., Athey, S.N., Lynch, J.M., De Frond, H., Gray, A., Jones, O.A.H., Brander, S., Steele, C., Moore, S., Sanchez, A., Nel, H., 2020. Reporting Guidelines to Increase the Reproducibility and Comparability of Research on Microplastics. Appl. Spectrosc. 74, 1066–1077. https://doi.org/10.1177/0003702820930292

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, A.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111, 10239–10244. https://doi.org/10.1073/pnas.1314705111

Cramer, W., Guiot, J., Fader, M., Garrabou, J., Gattuso, J.-P., Iglesias, A., Lange, M.A., Lionello, P., Llasat, M.C., Paz, S., Peñuelas, J., Snoussi, M., Toreti, A., Tsimplis, M.N., Xoplaki, E., 2018. Climate change and interconnected risks to sustainable development in the Mediterranean. Nat. Clim. Chang. 8, 972–980. https://doi.org/10.1038/s41558-018-0299-2

Crespo, M., Solé, M., 2016. The use of juvenile *Solea solea* as sentinel in the marine platform of the Ebre Delta: in vitro interaction of emerging contaminants with the liver detoxification system. Environ. Sci. Pollut. Res. 23, 19229–19236. https://doi.org/10.1007/s11356-016-7146-7

Cristo, M., 1998. Feeding ecology of *Nephrops norvegicus* (Decapoda: Nephropidae). J. Nat. Hist. 32, 1493–1498.

Cristo, M., Cartes, J.E., 1998. A comparative study of the feeding ecology of *Nephrops norvegicus* L. (Decapoda: Nephropidae) in the bathyal Mediterranean and the adjacent Atlantic. Sci. Mar. 62, 81–90. https://doi.org/10.3989/scimar.1998.62s181

Crosnier, A., Forest, J., 1973. Les Crevettes Profondes de L'Atlantique Oriental Tropical, in: Faune Tropicale. Mason, PAris, pp. 119–409.

Cundell, A.M., 1973. Plastic materials accumulating in Narragansett Bay. Mar. Pollut. Bull. 4, 187–188. https://doi.org/10.1016/0025-326X(73)90226-9

D'Onghia, G., Maiorano, P., Capezzuto, F., Carlucci, R., Battista, D., Giove, A., Sion, L., Tursi, A., 2009. Further evidences of deep-sea recruitment of *Aristeus antennatus* (Crustacea: Decapoda) and its role in the population renewal on the exploited bottoms of the Mediterranean. Fish. Res. 95, 236–245. https://doi.org/10.1016/j.fishres.2008.09.025

de Barros, M.S.F., dos Santos Calado, T.C., de Sá Leitão Câmara de Araújo, M., 2020. Plastic ingestion lead to reduced body condition and modified diet patterns in the rocky shore crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae). Mar. Pollut. Bull. 156, 111249. https://doi.org/10.1016/j.marpolbul.2020.111249

de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the Western Mediterranean Sea. Mar. Pollut. Bull. 140, 523–535. https://doi.org/10.1016/j.marpolbul.2019.01.053

Demestre, M., Cortadellas, N., Durfort, M., 1997. Ultrastructure of the sperm of the deep-sea decapod *Aristeus antennatus*. J. Morphol. 234, 79–87. https://doi.org/10.1002/(SICI)1097-4687(199710)234:1<79::AID-JMOR7>3.0.CO;2-I

Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44, 842–852.

Devriese, L.I., De Witte, B., Vethaak, A.D., Hostens, K., Leslie, H.A., 2017. Bioaccumulation of PCBs from microplastics in Norway lobster (*Nephrops norvegicus*): An experimental study. Chemosphere 186, 10–16. https://doi.org/10.1016/j.chemosphere.2017.07.121

Duncan, E., Botterell, Z., Broderick, A., Galloway, T., Lindeque, P., Nuno, A., Godley, B., 2017. A global review of marine turtle entanglement in anthropogenic debris: a baseline for further action. Endanger. Species Res. 34, 431–448. https://doi.org/10.3354/esr00865

EMODnet Bathymetry Consortium, 2020. EMODnet Digital Bathymetry (DTM 2020). EMODnet Bathymetry Consort. https://doi.org/https://doi.org/10.12770/bb6a87dd-e579-4036-abe1-e649cea9881a

Enders, K., Lenz, R., Beer, S., Stedmon, C.A., 2017. Extraction of microplastic from biota: Recommended acidic digestion destroys common plastic polymers. ICES J. Mar. Sci. 74, 326–331. https://doi.org/10.1093/icesjms/fsw173

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS One 9, e111913. https://doi.org/10.1371/journal.pone.0111913

Feist, S.W., Stentiford, G.D., Kent, M.L., Ribeiro Santos, A., Lorance, P., 2015. Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean. Mar. Environ. Res. 106, 42–50. https://doi.org/10.1016/j.marenvres.2015.02.004

Fischer, V., Elsner, N.O., Brenke, N., Schwabe, E., Brandt, A., 2015. Plastic pollution of the Kuril–Kamchatka Trench area (NW pacific). Deep Sea Res. Part II Top. Stud. Oceanogr. 111, 399–405. https://doi.org/10.1016/j.dsr2.2014.08.012

Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631–632, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046

Fossi, M.C., Pedà, C., Ferrer, M.C., Tsangaris, C., Mascaró, C.A., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2017. Biondicators for monitoring marine litter ingestion and impacts on Mediterranean biodiversity. Environ. Pollut. https://doi.org/10.1016/j.envpol.2017.11.019

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull. 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022

Fulton, M.H., Key, P.B., 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ. Toxicol. Chem. 20, 37–45. https://doi.org/10.1002/etc.5620200104

Galgani, F., Jaunet, S., Campillo, A., Guenegen, X., His, E., 1995. Distribution and abundance of debris on the continental shelf of the north-western Mediterranean Sea. Mar. Pollut. Bull. 30, 713–717. https://doi.org/10.1016/0025-326X(95)00055-R

Galgani, F., Souplet, A., Cadiou, Y., 1996. Accumulation of debris on the deep sea floor off the French Mediterranean coast. Mar. Ecol. Ser. 142, 225–234. https://doi.org/10.3354/meps142225

Galimany, E., Marco-Herrero, E., Soto, S., Recasens, L., Lombarte, A., Lleonart, J., Abelló, P., Ramón, M., 2019. Benthic marine litter in shallow fishing grounds in the NW Mediterranean Sea. Waste Manag. 95, 620–627. https://doi.org/10.1016/j.wasman.2019.07.004

Galloway, T.S., Brown, R.J., Browne, M.A., Dissanayake, A., Lowe, D., Jones, M.B., Depledge, M.H., 2004. A Multibiomarker Approach to Environmental Assessment. Environ. Sci. Technol. 38, 1723–1731. https://doi.org/10.1021/es030570

García-Rivera, S., Lizaso, J.L.S., Millán, J.M.B., 2018. Spatial and temporal trends of marine litter in the Spanish Mediterranean seafloor. Mar. Pollut. Bull. 137, 252–261. https://doi.org/10.1016/j.marpolbul.2018.09.051

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, 19–24. https://doi.org/10.1126/sciadv.1700782

Goldberg, E.D., 1997. Plasticizing the seafloor: An overview. Environ. Technol. (United Kingdom) 18, 195–201. https://doi.org/10.1080/09593331808616527

Gorelli, G., Company, J.B., Bahamón, N., Sardà, F., 2017. Improving codend selectivity in the fishery of the deep-sea red shrimp *Aristeus antennatus* in the northwestern Mediterranean Sea. Sci. Mar. 81, 381. https://doi.org/10.3989/scimar.04575.25A

Gregory, M.R., 2009. Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philos. Trans. R. Soc. B Biol. Sci. 364, 2013–2025. https://doi.org/10.1098/rstb.2008.0265

Hara, J., Frias, J., Nash, R., 2020. Quantification of microplastic ingestion by the decapod crustacean *Nephrops norvegicus* from Irish waters. Mar. Pollut. Bull. 152, 110905. https://doi.org/10.1016/j.marpolbul.2020.110905

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297

Heyerdahl, H., 1971. Atlantic Ocean pollution and biota observed by the Ra Expeditions. Biol. Conserv. 3, 164–167.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environ. Sci. Technol. 46, 3060–75. https://doi.org/10.1021/es2031505

Hodgson, D.J., Bréchon, A.L., Thompson, R.C., 2018. Ingestion and fragmentation of plastic carrier bags by the amphipod *Orchestia gammarellus*: Effects of plastic type and fouling load. Mar. Pollut. Bull. 127, 154–159. https://doi.org/10.1016/j.marpolbul.2017.11.057

Holmström, A., 1975. Plastic films on the bottom of the Skagerrak. Nature 255, 622–623.

International Organization for Standardization, 2021. Plastics - Vocabulary (ISO 472: 2013) [WWW Document]. URL https://www.iso.org/obp/ui/#iso:std:iso:472:ed-4:v1:en (accessed 7.3.21).

Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science (80-.). 293, 629–637. https://doi.org/10.1126/science.1059199

Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of Virgin Micro- and Nanoplastics on Fish: Trends, Meta-Analysis, and Perspectives. Environ.

Sci. Technol. 54, 4733–4745. https://doi.org/10.1021/acs.est.9b05995

Jakob, E.M., Marshall, S.D., Uetz, G.W., 1996. Estimating Fitness: A Comparison of Body Condition. Oikos 77, 61–67.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/10.1126/science.1260352

Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., Hwang, U.K., Zhou, B., Souissi, S., Lee, S.J., Lee, J.S., 2016. Microplastic Size-Dependent Toxicity, Oxidative Stress Induction, and p-JNK and p-p38 Activation in the Monogonont Rotifer (*Brachionus koreanus*). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01441

Johnson, M.L., Johnson, M., 2013. The ecology and biology of *Nephrops norvegicus*. Advances in Marine Biology, London, UK. https://doi.org/10.2307/3523

Jones, P.L., Obst, J.H., 2000. Effects of starvation and subsequent refeeding on the size and nutrient content of the hepatopancreas of *Cherax destructor* (Decapoda: Parastacidae). J. Crustac. Biol. 20, 431–441.

Kaandorp, M.L.A., Dijkstra, H.A., Van Sebille, E., 2020. Closing the Mediterranean Marine Floating Plastic Mass Budget: Inverse Modeling of Sources and Sinks. Environ. Sci. Technol. 54, 11980–11989. https://doi.org/10.1021/acs.est.0c01984

Kapiris, K., Thessalou-Legaki, M., 2011. Feeding ecology of the deep-water blue-red shrimp Aristeus antennatus (Decapoda: Aristeidae) in the Greek Ionian Sea (E. Mediterranean). J. Sea Res. 65, 151–160. https://doi.org/10.1016/j.seares.2010.09.005

Käppler, A., Windrich, F., Löder, M.G.J., Malanin, M., Fischer, D., Labrenz, M., Eichhorn, K.J., Voit, B., 2015. Identification of microplastics by FTIR and Raman microscopy: a novel silicon filter substrate opens the important spectral range below 1300 cm(-1) for FTIR transmission measurements. Anal. Bioanal. Chem. 407, 6791–6801. https://doi.org/10.1007/s00216-015-8850-8

Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hassellöv, M., Leslie, H.A., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and microplastic accumulation. Mar. Pollut. Bull. 122, 403–408. https://doi.org/10.1016/j.marpolbul.2017.06.081

Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environ. Sci. Technol. 50, 3315–3326. https://doi.org/10.1021/acs.est.5b06069

Koelmans, A.A., Besseling, E., Foekema, E.M., 2014. Leaching of plastic additives to marine organisms. Environ. Pollut. 187, 49–54. https://doi.org/10.1016/j.envpol.2013.12.013

Koenig, S., Fernández, P., Company, J.B., Huertas, D., Solé, M., 2013. Are deep-sea organisms dwelling within a submarine canyon more at risk from anthropogenic contamination than those from the adjacent open slope? A case study of Blanes canyon (NW Mediterranean). Prog. Oceanogr. 118, 249–259. https://doi.org/10.1016/j.pocean.2013.07.016

Kühn, S., van Franeker, J.A., 2020. Quantitative overview of marine debris ingested by marine megafauna. Mar. Pollut. Bull. 151, 110858. https://doi.org/10.1016/j.marpolbul.2019.110858

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Lagardère, J.P., 1977. Recherches sur la distribution verticale et sur l'alimentation des crustacés decapodes benthiques de la Pente Continentale de l'Atlantique Nord Oriental (Golfe de Gascogne et Maroc). Analyse des groupements carcinologiques. Bull. du Cent. d'Etudes Rech. Sci. Biarritz 11, 367–440.

Laist, D.W., 1987. Overview of the Biological Effects of Lost and Discarded Plastic Debris in the MArine Environment. Mar. Pollut. Bull. 18, 319–326.

Lakshmi Kavya, A.N. V, Sundarrajan, S., Ramakrishna, S., 2020. Identification and characterization of micro-plastics in the marine environment: A mini review. Mar. Pollut. Bull. 160, 111704. https://doi.org/10.1016/j.marpolbul.2020.111704

Law, K.L., Morét-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M., 2010. Plastic Accumulation in the North Atlantic Subtropical Gyre. Science (80-.). 329, 1185–1189. https://doi.org/10.1126/science.1192321

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103

Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. Proc. Natl. Acad. Sci. 113, E4121–E4122. https://doi.org/10.1073/pnas.1606615113

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in mussels along the coastal waters of China. Environ. Pollut. 214, 177–184. https://doi.org/10.1016/j.envpol.2016.04.012

Liubartseva, S., Coppini, G., Lecci, R., Clementi, E., 2018. Tracking plastics in the Mediterranean: 2D Lagrangian model. Mar. Pollut. Bull. 129, 151–162. https://doi.org/10.1016/j.marpolbul.2018.02.019

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G

Manan, H., Zhong, J.M.H., Othman, F., Ikhwanuddi, M., 2015. Histopathology of the

Hepatopancreas of Pacific White Shrimp, *Penaeus vannamei* from None Early Mortality Syndrome (EMS) Shrimp Ponds. J. Fish. Aquat. Sci. 10, 562–568. https://doi.org/10.3923/jfas.2015.562.568

Marcogliese, D.J., 2005. Parasites of the superorganism: Are they indicators of ecosystem health? Int. J. Parasitol. 35, 705–716. https://doi.org/10.1016/j.ijpara.2005.01.015

Markic, A., Gaertner, J.C., Gaertner-Mazouni, N., Koelmans, A.A., 2020. Plastic ingestion by marine fish in the wild. Crit. Rev. Environ. Sci. Technol. 50, 657–697. https://doi.org/10.1080/10643389.2019.1631990

MedECC, 2020. Climate and Environmental Change in the Mediterranean Basin - Current Situation and Risks for the Future. First Mediterranean Assessment Report. Union for the Mediterranean, PLan Bleu, UNEP/MAP, Marseille, France. https://doi.org/10.5281/zenodo.4768833

Meeker, J.D., Sathyanarayana, S., Swan, S.H., 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos. Trans. R. Soc. B Biol. Sci. 364, 2097–2113. https://doi.org/10.1098/rstb.2008.0268

Micheli, F., Halpern, B.S., Walbridge, S., Ciriaco, S., Ferretti, F., Fraschetti, S., Lewison, R., Nykjaer, L., Rosenberg, A.A., 2013. Cumulative human impacts on Mediterranean and Black Sea marine ecosystems: Assessing current pressures and opportunities. PLoS One 8. https://doi.org/10.1371/journal.pone.0079889

Miller, M.E., Hamann, M., Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. PLoS One 15, e0240792. https://doi.org/10.1371/journal.pone.0240792

Moore, C.J., Moore, S.L., Leecaster, M.K., Weisberg, S.B., 2001. A comparison of plastic and plankton in the North Pacific Central Gyre. Mar. Pollut. Bull. 42, 1297–1300. https://doi.org/10.1016/S0025-326X(01)00114-X

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032

Napper, I.E., Thompson, R.C., 2020. Plastic Debris in the Marine Environment: History and Future Challenges. Glob. Challenges 4, 1900081. https://doi.org/10.1002/gch2.201900081

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M., Johns, D.G., 2019. The rise in ocean plastics evidenced from a 60-year time series. Nat. Commun. 10, 1622. https://doi.org/10.1038/s41467-019-09506-1

Pagter, E., Frias, J., Kavanagh, F., Nash, R., 2020. Differences in microplastic abundances within demersal communities highlight the importance of an ecosystem-based approach to microplastic monitoring. Mar. Pollut. Bull. 160, 111644. https://doi.org/10.1016/j.marpolbul.2020.111644

Palanques, A., Lopez, L., Guillén, J., Puig, P., Masqué, P., 2017. Decline of trace metal pollution in the bottom sediments of the Barcelona City continental shelf (NW Mediterranean). Sci. Total Environ. 579, 755–767. https://doi.org/10.1016/j.scitotenv.2016.11.031

Patwardhan, S.S., 1935. On the Structure and Mechanism of the Gastric Mill in Decapoda. VI. The Structure of the Gastric Mill in Natantous Macrura* - Penaeidea and Stenopidea; Conclusion. Proc. Indian Acad. Sci. - Sect. B 2, 155–174.

Patwardhan, S.S., 1934. On the Structure and Mechanism of the Gastric Mill in Decapoda. IV. The Structure of the Gastric Mill in Reptantous Macrura. Proc. Indian Acad. Sci. - Sect. B 1, 183–196. https://doi.org/10.1007/BF03049344

Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Bravo Rebolledo, E.L., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Anal. Methods Methods 9, 1454–1469. https://doi.org/10.1039/C6AY02419J

Reboul, P., 1997. Pioneers of plastics. Mater. World 5, 720–722. https://doi.org/10.1179/isr.1998.23.2.169

Renner, G., Schmidt, T.C., Schram, J., 2018. Analytical methodologies for monitoring micro(nano)plastics: Which are fit for purpose? Curr. Opin. Environ. Sci. Heal. 1, 55–61. https://doi.org/10.1016/j.coesh.2017.11.001

Rice, A.L., Chapman, C.J., 1971. Observations on the burrows and burrowing behaviour of two mud-dwelling decapod crustaceans, *Nephrops norvegicus* and *Goneplax rhomboides*. Mar. Biol. Int. J. Life Ocean. Coast. Waters 10, 330–342. https://doi.org/10.1007/BF00368093

Rist, S., Carney Almroth, B., Hartmann, N.B., Karlsson, T.M., 2018. A critical perspective on early communications concerning human health aspects of microplastics. Sci. Total Environ. 626, 720–726. https://doi.org/10.1016/j.scitotenv.2018.01.092

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC - Trends Anal. Chem. 65, 47–53. https://doi.org/10.1016/j.trac.2014.10.011

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711. https://doi.org/10.1002/etc.4371

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 1–7. https://doi.org/10.1038/srep03263

Ruiz-Orejón, L.F., Sardá, R., Ramis-Pujol, J., 2018. Now, you see me: High concentrations of floating plastic debris in the coastal waters of the Balearic Islands (Spain). Mar. Pollut. Bull. 133, 636–646. https://doi.org/10.1016/j.marpolbul.2018.06.010

Ryan, P.G., 2018. Entanglement of birds in plastics and other synthetic materials. Mar. Pollut. Bull. 135, 159–164. https://doi.org/10.1016/j.marpolbul.2018.06.057

Ryan, P.G., Moloney, C.L., 1990. Plastic and other artefacts on South African beaches: temporal trends in abundance and composition. S. Afr. J. Sci. 86, 450–452.

Sanchez-Vidal, A., Thompson, R.C., Canals, M., de Haan, W.P., 2018. The imprint of microfibres in southern European deep seas. PLoS One 13, e0207033. https://doi.org/10.1371/journal.pone.0207033

Sardà, F., Demestre, M., 1985. Determination of the intermoult stages in *Aristeus antennatus* (Risso, 1816) by setal development. Rapp. Comm. Int. Mer Méditerranée 29, 305–308.

Schuyler, Q., Hardesty, B.D., Wilcox, C., Townsend, K., 2012. To Eat or Not to Eat? Debris Selectivity by Marine Turtles. PLoS One 7, e40884. https://doi.org/10.1371/journal.pone.0040884

Shahidul Islam, M., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Mar. Pollut. Bull. 48, 624–649. https://doi.org/10.1016/j.marpolbul.2003.12.004

Silva, A.B., Bastos, A.S., Justino, C.I.L., da Costa, J.P., Duarte, A.C., Rocha-Santos, T.A.P., 2018. Microplastics in the environment: Challenges in analytical chemistry - A review. Anal. Chim. Acta 1017, 1–19. https://doi.org/10.1016/j.aca.2018.02.043

Solé, M., Antó, M., Baena, M., Carrassón, M., Cartes, J.E., Maynou, F., 2010a. Hepatic biomarkers of xenobiotic metabolism in eighteen marine fish from NW Mediterranean shelf and slope waters in relation to some of their biological and ecological variables. Mar. Environ. Res. 70, 181–188. https://doi.org/10.1016/j.marenvres.2010.04.008

Solé, M., Baena, M., Arnau, S., Carrasson, M., Maynou, F., Cartes, J.E., 2010b. Muscular cholinesterase activities and lipid peroxidation levels as biomarkers in several Mediterranean marine fish species and their relationship with ecological variables. Environ. Int. 36, 202–211. https://doi.org/10.1016/j.envint.2009.11.008

Sreeram, M.P., Menon, N.R., 2005. Histopathological changes in the hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni* exposed to petroleum hydrocarbons. J. Mar. Biol. Assoc. India 47, 160–168.

Stentiford, G.D., Longshaw, M., Lyons, B.P., Jones, G., Green, M., Feist, S.W., 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar. Environ. Res. 55, 137–159. https://doi.org/10.1016/S0141-1136(02)00212-X

Stentiford, G.D., Neil, D.M., 2011. Diseases of *Nephrops* and *Metanephrops*: A review. J. Invertebr. Pathol. 106, 92–109. https://doi.org/10.1016/j.jip.2010.09.017

Suaria, G., Aliani, S., 2014. Floating debris in the Mediterranean Sea. Mar. Pollut. Bull. 86, 494–504. https://doi.org/10.1016/j.marpolbul.2014.06.025

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 37551. https://doi.org/10.1038/srep37551

Suman, K.H., Haque, M.N., Uddin, M.J., Begum, M.S., Sikder, M.H., 2021. Toxicity and biomarkers of micro-plastic in aquatic environment: a review. Biomarkers 26, 13–25. https://doi.org/10.1080/1354750X.2020.1863470

Taylor, M.L., Gwinnett, C., Robinson, L.F., Woodall, L.C., 2016. Plastic microfibre ingestion by deep-sea organisms. Sci. Rep. 6, 1–9. https://doi.org/10.1038/srep33997

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, J., John, A.W.G., McGonigie, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science (80-.). 304, 838. https://doi.org/10.1126/science.1094559

Tubau, X., Canals, M., Lastras, G., Rayo, X., Rivera, J., Amblas, D., 2015. Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: The role of hydrodynamic processes. Prog. Oceanogr. 134, 379–403. https://doi.org/10.1016/j.pocean.2015.03.013

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64, 178–189. https://doi.org/10.1016/j.ecoenv.2005.03.013

van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.-O., Pedersen, J., Stienen, E.W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North Sea. Environ. Pollut. 159, 2609–2615. https://doi.org/10.1016/j.envpol.2011.06.008

Vogt, G., Quinitio, E.T., Pascual, F.P., 1986. *Leucaena leucocephala* leaves in formulated feed for *Penaeus monodon*: a concrete example of the application of histology in nutrition research. Aquaculture 59, 209–234. https://doi.org/10.1016/0044-8486(86)90005-0

Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of Plastic Microfibers by the Crab *Carcinus maenas* and Its Effect on Food Consumption and

Energy Balance. Environ. Sci. Technol. 49, 14597–14604. https://doi.org/10.1021/acs.est.5b04026

Welden, N.A.C., Cowie, P.R., 2016a. Degradation of common polymer ropes in a sublittoral marine environment. Mar. Pollut. Bull. 118, 248–253. https://doi.org/10.1016/j.marpolbul.2017.02.072

Welden, N.A.C., Cowie, P.R., 2016b. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ. Pollut. 218, 895–900. https://doi.org/10.1016/j.envpol.2016.08.020

Welden, N.A.C., Cowie, P.R., 2016c. Environment and gut morphology influence microplastic retention in langoustine, *Nephrops norvegicus*. Environ. Pollut. 214, 859–865. https://doi.org/10.1016/j.envpol.2016.03.067

Wilder, S.M., Raubenheimer, D., Simpson, S.J., 2016. Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. Funct. Ecol. 30, 108–115. https://doi.org/10.1111/1365-2435.12460

Winston, G.W., Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19, 137–161. https://doi.org/10.1016/0166-445X(91)90033-6

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317–140317. https://doi.org/10.1098/rsos.140317

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013a. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013b. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P., Yonan, Y., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17. https://doi.org/10.1016/j.ancene.2016.01.002

Zariquey Alvarez, R., 1968. Crustáceos decápodos ibéricos. Investig. Pesq. 32, 510.