



## Flotsam, an overlooked vector of alien dispersal from ports

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### ARTICLE INFO

#### Keywords:

Marine litter  
Non-indigenous species  
Species dispersal  
Community diversity

### ABSTRACT

Anthropogenic litter is considered a potential vector for the dispersal of non-indigenous species (NIS) in marine ecosystems. Using the bay of Gijón (Southwestern Bay of Biscay) as a case study, we studied the composition and potential transfer of the communities inhabiting three different environment components: 1) natural and artificial substrates from the international port of Gijón, 2) six proximate rocky beaches and 3) floating litter collected in the adjacent coast. A total of 717 organisms were morphologically identified and DNA barcoded using COI and 18S genes. In total 23 NIS were detected, six of them considered invasive in the area. The taxonomic profiles of the three environment components were significantly different, flotsam containing higher proportions of Hexanauplia and less mollusks, echinoderms and polychaetes than ports and beaches. Contrary to expectations, floating litter showed higher densities of native and exotic species than beaches or port surfaces. This and shared haplotypes between port, flotsam and beaches in some invasive species may indicate that marine litter could represent a new habitat for species to disperse into new areas.

### 1. Introduction

Marine ecosystems are a precious asset for humans since they are an important source of goods and services (FAO, 2020; Datta et al., 2015; Spalding et al., 2014; Cusack et al., 2021). In a scenario of increasing anthropic pressures such as overfishing, habitat destruction and pollution, marine ecosystems are facing a global degradation (Halpern et al., 2015). This problem has been well assessed in Europe, where human pressures affect practically the entire marine environment (Korpinen et al., 2020). Another important factor that has been gaining importance over the years and that is nowadays considered a major threat for marine ecosystems is marine anthropogenic litter, which is defined as “any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment” (UNEP, 2009). This anthropogenic litter is another crucial factor that affects marine ecosystems and is one of the major obstacles to achieve the Sustainable

Development Goal (SDG) #14 “Life under water”, that aims to conserve and sustainably use the oceans, seas and marine resources (Goal 14, United Nations Sustainable Development Goals). Synthetic polymers (commonly known as plastics) have become a recognized global environmental problem. It is estimated that approximately 8 million metric tons of plastic enter the marine environment annually (Jambeck et al., 2015) and the production is continuously increasing (Geyer et al., 2017).

This situation is the cause of the severe environmental damages in marine ecosystems that harm aquatic organisms and endangers marine biodiversity (Deudero & Alomar, 2015; Fossi et al., 2018), consequently affecting the sustainability of fishing resources worldwide, from the Baltic (Depellegrin et al., 2020) to Oceania (Smith, 2012) and Africa (Masiá et al., 2022). After entering the marine environment, floating litter can easily be ingested or entangled by the inhabiting species, increasing their mortality (Steer et al., 2017; Rizzi et al., 2019). Also,

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when breaking in smaller fragments like microplastics (those plastic fragments smaller than 5 mm long), plastic litter can release chemical substances or even accumulate persistent organic pollutants and other substances of concern that can be transferred to the food web (Hahladakis et al., 2018; Chen et al., 2019).

In addition to its adverse effects as a macro-pollutant, marine litter is a vector of biopollution as well. Biopollution (i.e., harmful and invasive species) poses a threat to native diversity in all oceans (Galgani et al., 2015; Ryan, 2015; Williams and Rangel-Buitrago, 2019; Occhipinti-Ambrogi, 2021) as species which are non-native to an ecosystem (known as exotic species, alien species or non-indigenous species) can establish new populations and cause severe impacts on these new habitats. At this point, following the terminology from Iannone et al. (2021), the classification of these species is modified from exotic to invasive species. Flotsam litter could be seen as a temporary niche of biopollutant species that move from one to another location attached to those light artificial substrates that can be passively transported far away (Kiessling et al., 2015; Wichmann et al., 2019; Maclean et al., 2021), even to remote islands (Rech et al., 2018). In fact, the role of flotsam to increase connectivity between islands is well known; some non-indigenous species (NIS) travel long distances, even crossing oceans, attached to floating litter (Miller et al., 2018; Therriault et al., 2018; García-Gómez et al., 2021). In this scenario, marine litter has been recommended to be classified as a “transport-stowaway” pathway in the Convention on the Biological Diversity Pathway Classification framework as it can help fouling species to spread rapidly using marine currents (Pergl et al., 2020).

Urban areas and maritime ports are of special interest because they are main exporters of marine litter to open waters (Barnes et al., 2009; Chen, 2015; Rayon-Viña et al., 2022). Simultaneously, maritime ports are hubs of exotic species that are mainly introduced by maritime traffic (Molnar et al., 2008) and due to the high litter density, can be used as a key location for biopollutants to disperse to other areas. Rech et al. (2016) warned about the likelihood of port-exported flotsam to be a key contributor of invasive species dispersal worldwide. Garcia-Vazquez et al. (2018) found that litter concentrated nearby port areas at the entry of the Baltic Sea contains a higher proportion of non-indigenous species than litter deposited on farther beaches. In the Cantabrian Sea, invasive species attached to beached plastic bottles and ropes are more abundant near ports (Miralles et al., 2018a) and eDNA biota profiles found in beached marine litter show clear similarities with the biota inhabiting ports (Ibabe et al., 2020).

Despite these evidences, the role of marine litter as a vector of NIS between ports and surrounding natural areas is still unclear. It is possible that invasive species restricted to ports, especially if they have limited mobility, or if surrounding areas exhibit sufficient native biodiversity for aliens might not be able to find empty niches to colonize (Shea and Chesson, 2002; Knight and Reich, 2005; Crooks et al., 2011). In recent years, different plans such as the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) have been produced to deal with the problem posed by marine litter. However, there is still a long road ahead in order to better understand the consequences of litter in the marine environment (either as a macropollutant or a vector for biopollutants).

In this work we studied the contribution of floating litter to the dispersal of invasive species, using the Southwestern Bay of Biscay as a case study. Macroscopic animals attached to natural and artificial substrates were sampled from three different environment components: 1) the main international port of the region, 2) floating objects collected outside the port in front of the adjacent coast, and 3) the six main rocky beaches in that part of the coast. DNA barcoding was used to identify biopollutants in the three environments, and the relative density and species sharing between environments to determine the role of flotsam in biopollution dispersal between the port and natural coastal areas. Expectations were: i) flotsam will act as a vector between ports and beaches; ii) only a fraction of biopollutants –those preferring plastic substrate– will be transferred from port to beaches; iii) consequently the

species density will decrease in the transfer vector (flotsam) in comparison to the donor (port) and recipient habitats (natural rocks).

## 2. Materials and methods

### 2.1. Ethics statement

This study was approved from the Research Ethics Committee of the Principality of Asturias with the reference 166/19. The general procedure of diving works was based on the Collective Agreement of Professional Diving and Hyperbaric Means (Ministerial Order of October 14, 1997, Spain), modified by the Collective Agreement of Professional Diving and Hyperbaric Means of October 18, 2016 accomplishing the directive UNE-EN 15333-1.

### 2.2. Study area

The study region selected was the Bay of Gijón (coordinates 43.573–43.551, –5.728 to –5.588; southwestern Bay of Biscay) and its surroundings. The international Port of Gijón has the local name of El Musel (Fig. 1), and has an adjacent marina between the port and Arbeyal beach. Beaches were selected for having a large surface of rocky substrate: Xivares, Arbeyal, El Rinconin, Peñarrubia, Cagonera and La Ñora (Fig. 1). Between Arbeyal and the city of Gijón there is another marina. Touristic sandy beaches were not considered since targets were species fouling on hard substrates. Structures of the port of Gijón including the marina, floating marine litter and rocks from the six beaches mentioned were sampled for attached biota. All samplings were done between January and July of 2017.

### 2.3. Sampling procedures

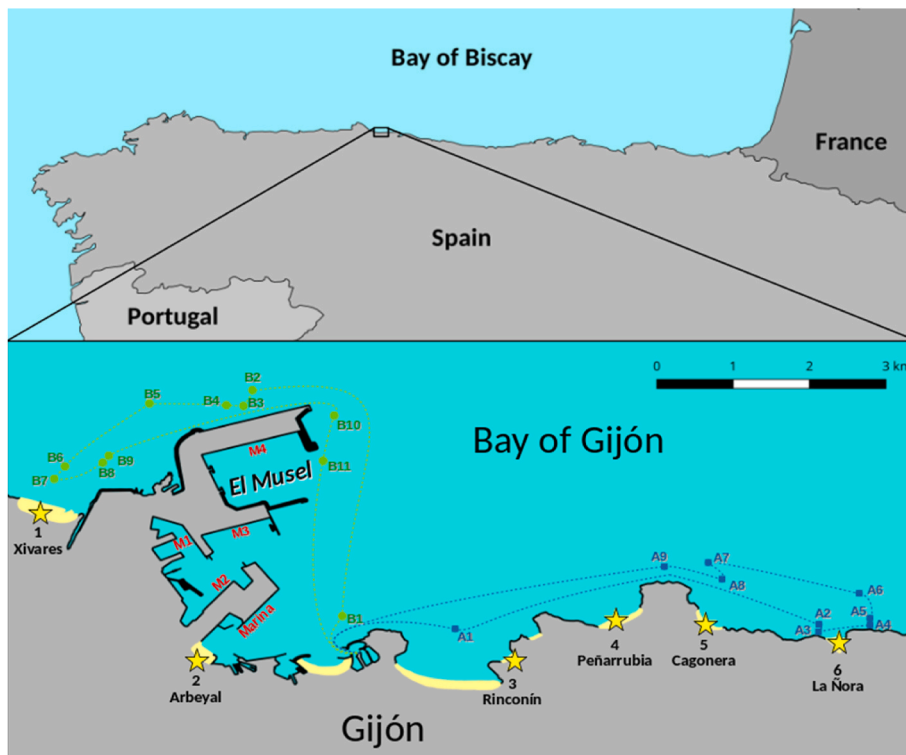
#### 2.3.1. Beach rocks and beached marine litter

In beaches, sampling was performed in transects on natural hard substrate (rocks, pebbles). Starting 30min before low tide, three perpendicular transects of 1m width from the water line to the green line (where vegetation starts) were deployed in each beach. These transects were separated evenly at least 100m one from each other to avoid confounding and to cover the whole surface of the beaches, whose surfaces differed greatly from each other. Therefore, the area sampled varied depending on the beach size being approximately between 300 and 400 m<sup>2</sup> per beach. The total surface sampled summing all beaches was approx. 2200 m<sup>2</sup>. All visible animals found inside transects were sorted morphologically by phenotype for taxonomic identification. Up to 10 individuals per phenotype (putative species) were selected for the genetic analysis and preserved in 100% ethanol for further DNA identification.

The beaches were sampled for beached marine litter too. During low tide, the items bigger than 2.5 cm found in the transects described above were collected, categorized by material and type of object (plastic, metal, glass, textile; ear buds, bags, bottles, cans, sanitary pads etc.) and photographed. Every item was checked for any visible macrobiota, fouling individuals were isolated, preserved in 100% ethanol and transported to the laboratory for further genetic identification. Litter objects were then thrown into recycle bins. The total surface of these litter items was approx. 42.5 m<sup>2</sup>. The surface estimation includes front and back surfaces of the materials. These samplings were performed between the 13th of January and the 27th of March, to avoid cleanings made by the administrations that could affect the results.

#### 2.3.2. Port sampling

The international Port of Gijón is the largest in the Southwestern Bay of Biscay. Sampling was carried out in four sections within the commercial port (two locations within each section) and in the adjacent marina (one location) in July 2017. Sampling procedure is described in detail in Miralles et al. (2021). Briefly, first a visual inspection was



**Fig. 1.** Map showing the locations sampled in this study within southwest Bay of Biscay. The sites where floating objects were collected are marked on two routes departing from Gijón marina (eastward A1-A9 and westward B1-B11). The sampled beaches, marked with yellow stars, are from 1 to 6: Xivares, Arbeyal, Rinconin, Peñarrubia, Cagonera and La Nora, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

conducted during low tide to locate biota patches and determine, by using quadrats, the different types of organisms attached to hard substrates, including rocky ground and concrete structures. To standardize the sampling effort, the surface sampled from each quadrat was approximately 200 m<sup>2</sup>. Macroscopic animals were sampled proportionally to their relative abundance for 30 min per location. Individuals were scraped using spatulas when necessary and then introduced in ambient water (contained in 10L buckets) after being separated by morphotype for posterior visual identifications. The total surface sampled from port surfaces was approximately 1800 m<sup>2</sup>. In addition, three quadrats of approx. 30 × 30cm were scratched from each of the three vessels (one of them arrived from Russia and the other two from Spanish ports) long-docked in the port to have a representation of all the artificial surfaces carrying organisms. These quadrats were placed in three different zones along the draft of the vessel: stern, mid-ship and bow. These samplings were done by professional divers and all the collected individuals were stored and then classified by morphotype for posterior visual identifications.

The individuals collected were transported to the laboratory in coolers. Once in the laboratory, individuals were sorted *de visu*, and preserved in 100% ethanol for further genetic identification.

### 2.3.3. Flotsam sampling

Two trips were performed in the waters of the bay of Gijón, one eastward and other westward (see Fig. 1), following the coastline and traveling at an average of 10 knots in July 2017. In each trip visual sightings of floating objects were made by four people on the deck of the vessel. Every flotsam item traced down was collected along with any associated solid material, its location registered with a GPS, isolated in individual empty bags and transported in coolers to the laboratory. In the laboratory, litter items were listed, measured, photographed and examined for macroscopic individuals fouling on or carried inside the object, that were picked and preserved in 100% ethanol in the laboratory of Genetics of Natural Resources at the University of Oviedo. A total of 45 objects were found (principally of plastic), summing in total approx. 2.8 m<sup>2</sup> of surface. As for beached litter, the surface estimation

included front and back surfaces of the materials.

## 2.4. Taxonomic analysis

### 2.4.1. Species identification from morphology

Visual identification based on morphological traits was performed following the Guide to Seashores and Shallow Seas (Campbell, 2005) to the lowest possible taxonomic level (species whenever possible; genera, family or order in some cases). The specimens were double-checked with the help of experts in the laboratory, using magnifying glasses when needed. Only those classified down to a species or genera level were retained for further analysis. The taxonomic nomenclature followed the World Register of Marine Species (WoRMS Editorial Board, 2020). The invasiveness capacity of the species was assessed according to the European Alien Species Information Network EASIN (Tsiamis et al., 2021), the Invasive Species Compendium CABI (2021), the Global Invasive Species Database (Pagad et al., 2015), and the native distributions as in WoRMS. Species were classified as native (those that occur naturally in a geographic area), NIS (those that do not occur naturally in a specific geographic area but do not cause any known impact; considered synonym of exotic, alien and nonnative) or invasive (those that are nonnative to a specific geographic area and cause environmental or economic harm or harm to humans) following Iannone et al. (2021).

### 2.4.2. Genetic analysis

To confirm the visual identification and perform haplotype analysis, DNA was extracted from small pieces of muscular tissues (approx. 2 mm<sup>3</sup>), following (Estoup et al., 1996) protocol. In the case of very small individuals, the full organism was used. For the individuals that were not successfully extracted following Estoup et al. (1996) we used E.Z.N. A® Mollusc DNA kit (Omega Bio-tek) following manufacturer's instructions.

DNA barcoding was done by PCR-amplifying a fragment of the cytochrome oxidase subunit I gene (COI) and 18S rDNA (18S). COI was amplified by using jgLCO1490 and jgHCO2198 primers and the PCR program and conditions detailed in Geller et al. (2013). For the

identification of the species attached to the beached litter COI and 18S rRNA genes were employed. 18S was amplified using 18S-EukF and 18S-EukR primers and conditions described in Medlin et al. (1988). For the polychaete species from these samples COI was preferred. PCR products were analyzed on 2% agarose gels stained with SimplySafe to confirm amplification before sequencing. Amplicons were quantified by using a Qubit dsDNA HS kit (ThermoFisher scientific, USA) and remaining primers and nucleotides were removed with Clean Sweep kit (ThermoFisher scientific, USA). Sequencing of the PCR products was performed principally in MacroGen company (MacroGen, 2017). Part of the sequences were obtained in the DNA Analysis Facility of Scientific Technical Services, Oviedo University using BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher scientific, USA) and an ABI Prism 3100xl Genetic Analyzer (ThermoFisher scientific, USA). Individuals from beached litter were forward and reverse sequenced and the rest only forward sequenced.

Final sequences were edited with SnapGene Viewer software and contrasted with BLAST software (Dumontier and Hogue, 2002) in NCBI (National Center of Biotechnology Information) within GenBank. The threshold for species assignment was a maximum E-value = 1e-100 and at least 97% nucleotide identity, which is considered the level at which species differ in the case of COI (Hebert et al., 2003) and 18S (Brown et al., 2016).

DnaSP v6 (Rozas et al., 2017) was used to obtain haplotypes from the sequences of each species. MEGA software version X (Kumar et al., 2018) was employed to reconstruct a Maximum Parsimony Tree using COI haplotypes from *Magallana gigas* and sequences from different regions downloaded from GenBank. A haplotype network was constructed from *Magallana gigas* sequences using the software Network 10.2.0.0 (available at <http://www.fluxus-technology.com>) applying default settings in Median Joining.

## 2.5. Statistics

Parametric or non-parametric tests were done after checking normality in the dataset using Shapiro-Wilk test. The number of species per phylum, the total number of species, and the proportion of NIS (NIS individuals over the total number of individuals, or NIS over the total number of species) were measured in each sample. To check differences between components (specifically, if biota is significantly different in flotsam, ports and beaches), first we looked at the distribution of species per phylum (i.e., the taxonomic diversity), then at the proportion of NIS. Comparison between environments for the distribution of NIS versus native species, and for the distribution of shared versus exclusive species, was done using contingency Chi-square analysis.

Next, we looked at the density of exclusive (species that were only found in one of the three environment components) and shared species (the ones that were found in different environment components). Species density was calculated as the number of species divided by the square meters sampled. Two-way ANOVA without replication was employed to test the effect of compartment (port, beaches, flotsam) and species type (exclusive versus shared) on the species density.

Communities were finally compared using Bray-Curtis distance and 9999 permutations for the presence (1)/absence (0) of each species (because the surface sampled from each environment was not the same), and a plot was constructed from nonmetric multidimensional scale analysis (NMDS), checking stress and  $r^2$  of axis 1 and 2.

PAST software by Hammer et al. (2001) was employed for statistical tests and construction of NMDS plots.

## 3. Results

### 3.1. Overview of animal biota in the three environment components

Animals sampled were taxonomically identified down to the species level whenever possible. Their DNA barcodes can be found in GenBank

with the accession numbers MT258923- MT258975 for samples from beaches and flotsam, and MN185333-MN185374, MN164033-MN164046 for port samples.

In Gijón port 427 individuals from 73 species were barcoded (Table 1): 20 from the marina, 56 from the commercial port and 15 attached to ship hulls (Supplementary Table 1). Port dataset details can be found in Miralles et al. (2021).

In beach transects a total of 218 organisms were taxonomically identified from morphology and confirmed from DNA, belonging to 54 different species. The number of species per beach ranged from 11 in Arbeyal to 29 in Rinconin (Table 1, Supplementary Table 1). Only a few macroscopic organisms (five individuals of 4 species) were found attached to beached litter, the great majority of ashore objects being apparently recently abandoned with no observable biota attached. Identification to species level from DNA barcoding confirmed the presence of acorn barnacle *Perforatus perforatus* (on a plastic in Cagonera), the tunicate *Didemnum vexillum* (also on Cagonera), the oyster *Neopycnodonte cochlear* (on hard plastic in Peñarrubia) and the polychaete *Sabellaria spinulosa* (on hard plastic in Rinconin).

Regarding floating marine litter (Fig. 2), 80% of the objects sampled (36 out of 45) carried visible fouling biota (Supplementary Table 2). We counted 67 macroscopic animals that could be visually observed on 19 of those objects (42.2%), representing a total of 26 species (Table 1, Supplementary Table 1); the rest of objects carried algae.

The species living on the three environment components did not exhibit the same taxonomic profile (Fig. 3), and the difference between them was highly significant ( $\chi^2 = 47.7$ , 22 d.f.,  $p = 0.001$ ). The community traveling on flotsam contained more Hexanauplia (barnacles) while less molluscs, echinoderms and polychaetes than ports and beaches. Considering the differences in surface sampled, Shannon diversity (Table 1) was not much different in the three different environment considered. The exception was the beached litter, not included as a habitat in this study, with a logical very low diversity.

Many species (31.5% of the total) were shared between the three components (Fig. 4): eight among the three environments, 20 between the port and beaches, five between the port and floating litter, and two between flotsam and beaches. In the port 54.8% of the species were exclusive (found only therein), while this percentage was 44.4% in beach rocks and flotsam.

The majority of species shared between environments belonged to five taxonomic groups that accounted for 28 of the 35 shared species (Supplementary Table 1): gastropods (9), bivalves (6), malacostraceans (5), polychaetes (4) and Hexanauplia –barnacles– (4). Then followed echinoderms (3), chitons (2), ascidians (1) and decapods (1).

**Table 1**

Summary of biota diversity in the three environment components considered (in bold), included the beached litter. Number of DNA-barcoded individuals in the sampling locations and litter (N); diversity as number of species (Species richness) and Shannon diversity index H (Shannon); number and proportion of non-indigenous individuals (%NIS individuals) and species (%NIS).

	N	Species richness	Shannon	NIS individuals (%)	NIS(%)
Xivares (st.1)	42	19	2.73	5 (11.9)	1 (5.3)
Arbeyal (st. 2)	22	11	2.31	6 (27.3)	2 (18.2)
Rinconin (st. 3)	48	29	3.27	2 (4.2)	2 (6.9)
Peñarrubia (st. 4)	29	20	2.95	1 (3.4)	1 (5)
Cagonera (st. 5)	33	17	2.74	0 (0)	0 (0)
La Ñora (st. 6)	34	16	2.64	2 (5.9)	1 (6.2)
<b>All beaches</b>	<b>218</b>	<b>54</b>	<b>3.62</b>	<b>16 (7.3)</b>	<b>4 (7.4)</b>
<b>Flotsam</b>	<b>67</b>	<b>26</b>	<b>3.00</b>	<b>7 (10.4)</b>	<b>6 (23.1)</b>
<b>Gijón port</b>	<b>427</b>	<b>73</b>	<b>3.65</b>	<b>100 (23.4)</b>	<b>21 (28.8)</b>
<b>Beached litter</b>	<b>5</b>	<b>4</b>	<b>1.33</b>	<b>1 (20)</b>	<b>1 (25)</b>





Fig. 2. Examples of floating objects sampled from point #21 (site B11 on westward route).

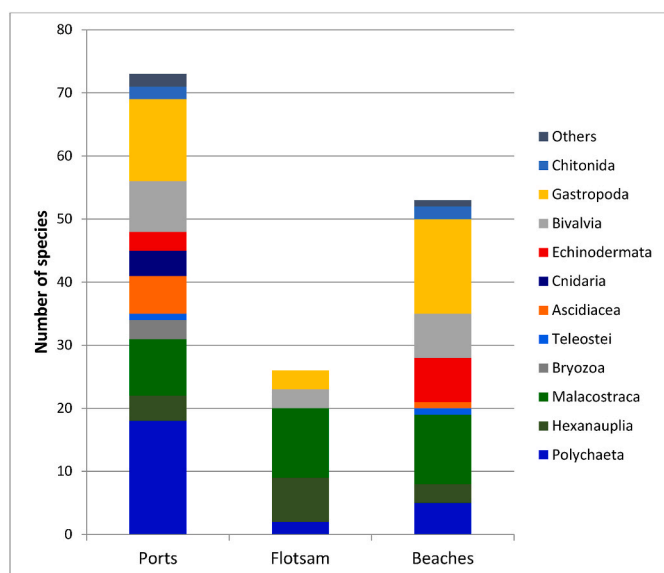


Fig. 3. Taxonomic profile of the species sampled from beaches, ports and floating litter.

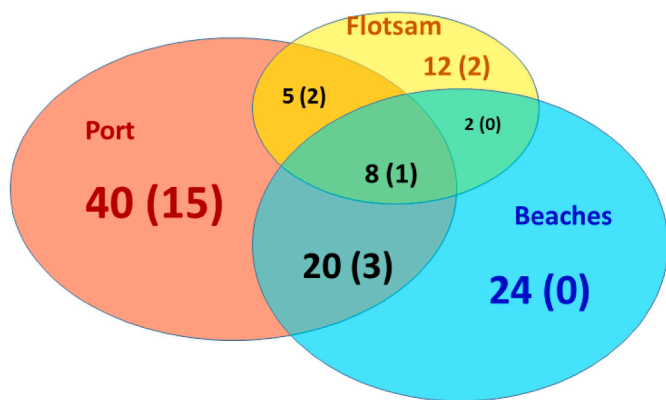


Fig. 4. Share of macroscopic animal species attached to hard substrate in the three environments analyzed. Number of species is given for each environment (from which NIS in parenthesis).

Considering the proportion of species of each big taxonomic group that occupy different environments (environment sharing), mollusks (53.1% of species), followed by echinoderms (42.9%) were by far the most shared (Table 2), followed by arthropods, annelids and chordates species that were shared by at least two environments in these samplings.

The distribution of species sharing environments (versus exclusive of an environment) in the considered taxonomic groups was significantly different ( $\chi^2 = 15.09$ , 7 d.f.,  $p = 0.03$ ), indicating that, in this study, the species of some taxonomic groups moved between environments (namely, port, flotsam and beaches) more frequently than the species of other groups.

Two-way ANOVA without replication (Table 3) revealed significant differences between environment components for species density ( $F_{2,5} = 140$ ,  $p = 0.007$ ). Litter flotsam -despite much smaller surface-contained similar species richness to that of individual beaches (Table 1), and its density of exclusive and shared species was 0.26 and 0.31 species/m<sup>2</sup> respectively. These densities were respectively 0.023 species/m<sup>2</sup> and 0.017 species/m<sup>2</sup> in the port, and 0.011 species/m<sup>2</sup> and 0.014 species/m<sup>2</sup> in beach rocks. This means that species density was ten-fold higher in flotsam than in hard substrates of beaches and in the port. On the other hand, the factor “species type by sharing” (shared versus exclusive of an environment) was not significant (Table 2), meaning that the density of shared and exclusive species was not significantly different in these environments.

### 3.2. Non-indigenous species in the ecosystem components analyzed

In total 124 individuals of 23 NIS were found and barcoded in this study (Table 4, Supplementary Table 1). They belonged to eight classes: Polychaeta, Ascidiacea (tunicates) and Bivalvia, with four species of each class; Hexanauplia (barnacles), Gymnolaemata (bryozoans) and Anthozoans (two sea anemones and one coral), with three species in each class; one Malacostraca (an isopod); one Actinopterygii (*Acanthogobius*, a fish). Six of the 23 NIS are invasive species (Table 4): two barnacles, three of the four tunicates, the fish and the Japanese oyster *Magallana gigas*.

The proportion of NIS (Tables 1 and 4, Fig. 4) in the three environments was significantly different ( $\chi^2 = 8.9$ , 2 d.f.,  $p = 0.01$  for the distribution of NIS versus native species). The port contained the highest proportion of NIS (28.8% of species), followed by flotsam (22.2% of species) and finally beaches (7.4% of species). Of the three environments, floating litter contained the highest density of NIS (Table 4), ten times higher than that of the port and 60 times higher than NIS density in beaches.

All the four NIS found from beaches were shared with ports (principally) and flotsam (Table 4). The majority of NIS of this study were found only within the port (15 species, the 65% of the total number of NIS). Two barnacles (*Austrominius modestus* and *Chamaesipho columna*) were found only on flotsam, and none was exclusive of beaches. A total of six NIS (the 26%) were shared between environments (Fig. 4, Table 4). Only one species, the Japanese oyster *Magallana gigas* (Supplementary Table 1), was present in the three environments considered (port, litter and natural substrate in beaches; Table 4). Different haplotypes were found for this species (Fig. 5). The most frequent Haplotype 1 was shared between the three environments (Fig. 5A). According to sequence databases, this haplotype and is also present in other regions (Fig. 5B) that are connected with the port of Gijón by maritime traffic: North America, North Sea and Mediterranean Sea (Miralles et al., 2018b). This would suggest transference of this species between environments, probably in the direction port – flotsam - beaches. Five more haplotypes were found only in beaches, which is expected given repeated introductions of *Magallana gigas* in the region via aquaculture (Semeraro et al., 2016).

Similar haplotype analysis could not be done in other species due to insufficient information, because the majority of the shared species had too few haplotypes. For example, *Balanus trigonus* sequences had the same haplotype in port and flotsam individuals, and *Mytilus trossulus* had only two haplotypes, one in the port and other in two individuals found from beaches, which is not much informative.

Focusing specifically on the invasive NIS, all of them were in the port except the acorn barnacle *Austrominius modestus* that was found only on

**Table 2**

Species occupying different environments (environment sharing) by taxonomic groups. The total number of species in each taxonomic group (NSp) and the number of species shared between different environments are shown. Regarding each taxonomic group, the proportion of species occupying more than one environment (%multi-habitat) is indicated.

	NSp	Environment sharing				% multi-environment
		Port & Beach	Port & Litter	Litter & Beach	All environments	
Annelida	22	2	2	0	0	0.182
Arthropoda	32	1	3	2	4	0.313
Bryozoa	3	0	0	0	0	0
Chordata	8	1	0	0	0	0.125
Cnidaria	4	0	0	0	0	0
Echinodermata	7	3	0	0	0	0.429
Mollusca	32	13	0	0	4	0.531
Other	3	0	0	0	0	0

**Table 3**

Two-way ANOVA without replication showing the effect of the factors environment type (litter, port, beaches) and species sharing (shared versus exclusive species) on the density of species in this study, measured as number of species per square meter.

Factor	Sum of squares	df	Mean square	F	p (same)
Environment type:	0.098	2	0.049	140	0.007
Sharing:	0.0003	1	0.0003	0.837	0.457
Error:	0.0007	2	0.0003		
Total:	0.099	5			

flotsam in these samplings (Table 4). The majority were restricted to the port with a few exceptions: *Balanus trigonus* (present in litter too), *Microcosmus squamiger* (also in beaches) and *Magallana gigas* (in the three environments as explained above). The proportion of species shared between environments, three of 6, was not very different to that of total NIS (7 of 23, 30.4%) and that of native species (29 of 88, 33%). From these data, NIS/invasive NIS do not move more frequently among environments than native species.

NMDS analysis (low stress of 0.011,  $r^2$  of axis 1 = 0.68 and  $r^2$  of axis 2 = 0.11) supported a possible role of flotsam as an intermediary between the port and surrounding beaches for species dispersal. The

scatter plot showed flotsam biota located between the port and the beaches (Fig. 6). Arbeyal and Xivares, the closest beaches to flotsam in the NMDS plot, are the closest beaches to the port (Fig. 1).

#### 4. Discussion

The first novelty of this study is the evidence of a higher density of species in flotsam than in port structures and natural substrates. At first, the opposite could be expected, because only a fraction of biopollutants (those preferring plastic substrate) would be transferred from port to beaches using flotsam as a vector. A high density from taxa that prefer plastic substrates could be expected on flotsam, as in the case of goose and acorn barnacles (Whitehead et al., 2011) or the bryozoan *Bugula neritina* (Li et al., 2016), but more species per surface unit was a really unexpected result, contradicting Hypothesis iii) that would expect a decrease in species density in the transfer vector (flotsam) in comparison to the donor (port) and recipient habitats (natural rocks). The reason could lie in a diversity of shapes, colors and materials in floating objects (illustrated in Fig. 2), that have sufficient space to shelter many small individuals of the same or different species. The sum of such heterogeneous objects under the denomination of “floating litter habitat” makes it a quite diverse environment.

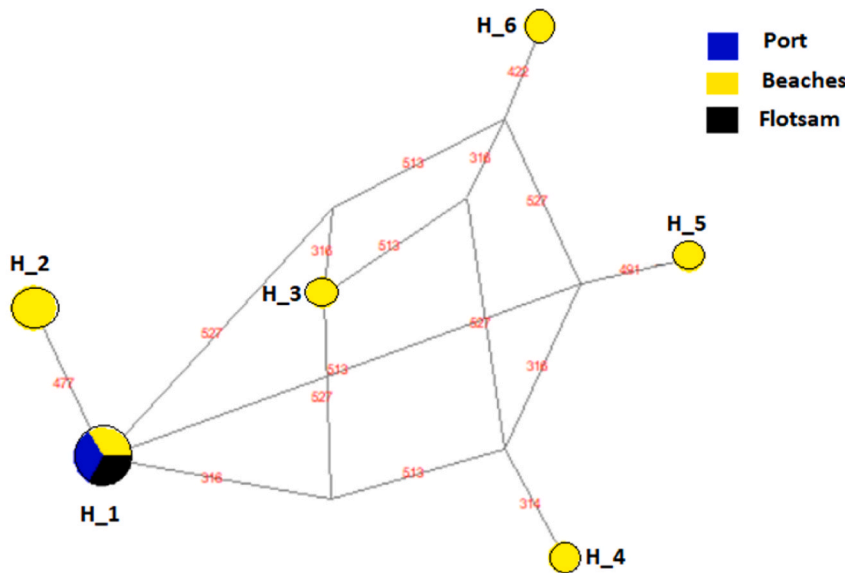
The results from this study showed the expected differences in the

**Table 4**

Non-indigenous species found in this study in the three environments analyzed, and their invasive status as recognized in the Invasive Species Compendium (CABI, 2021) and National Estuarine and Marine Exotic Species Information System (Fofonoff et al., 2018). N = number of individuals barcoded. The density of NIS in each environment is presented.

Species	Class	Native distribution	Status	N	Port	Flotsam	Beaches
<i>Phyllococe groenlandica</i>	Polychaeta	Other North Atlantic waters	Exotic	2	1	0	0
<i>Spirobranchus laticapus</i>	Polychaeta	Red Sea	Exotic	4	0.75	0.25	0
<i>Spirobranchus taeniatus</i>	Polychaeta	Australia	Exotic	2	0.5	0.5	0
<i>Dipolydora capensis</i>	Polychaeta	South Africa	Exotic	2	1	0	0
<i>Austrominius modestus</i>	Hexanauplia	Australasian	Invasive	2	0	1	0
<i>Balanus trigonus</i>	Hexanauplia	Pacific	Invasive	9	0.89	0.11	0
<i>Chamaesipho columna</i>	Hexanauplia	New Zealand	Exotic	1	0	1	0
<i>Livoneca redmanii</i>	Malacostraca	Caribbean	Exotic	5	1	0	0
<i>Bugula neritina</i>	Gymnolaemata	Unknown	Cryptogenic	1	1	0	0
<i>Amathia verticillata</i>	Gymnolaemata	Unknown	Cryptogenic	7	1	0	0
<i>Watersipora subtorquata</i>	Gymnolaemata	Unknown	Cryptogenic	12	1	0	0
<i>Acetrogobius cf. pflaumii</i>	Actinopterygii	North Pacific	Invasive	1	1	0	0
<i>Diplosoma listerianum</i>	Ascidacea	Unknown	Cryptogenic	3	1	0	0
<i>Microcosmus squamiger</i>	Ascidacea	Australia	Invasive	4	0.5	0	0.5
<i>Botryllus schlosseri</i>	Ascidacea	Unknown-Pacific?	Cryptogenic	1	1	0	0
<i>Styela plicata</i>	Ascidacea	Atlantic - Western central	Invasive	3	1	0	0
<i>Anthopleura anjunae</i>	Anthozoa	Indo-Pacific	Exotic	1	1	0	0
<i>Anthopleura elegantissima</i>	Anthozoa	Pacific	Exotic	2	1	0	0
<i>Caryophyllia grayi</i>	Anthozoa	South Pacific	Exotic	1	1	0	0
<i>Mytilaster minimus</i>	Bivalvia	East Mediterranean	Exotic	27	0.89	0	0.11
<i>Mytilus trossulus</i>	Bivalvia	Northwest Atlantic - Baltic	Exotic	7	0.71	0	0.29
<i>Magallana gigas</i>	Bivalvia	Northwest Pacific	Invasive	25	0.56	0.08	0.36
<i>Talochlamys multistriata</i>	Bivalvia	South Africa/Mediterranean	Exotic	2	1	0	0
			NIS number		21	6	4
			NIS density		0.012	0.132	0.002

A)



B)

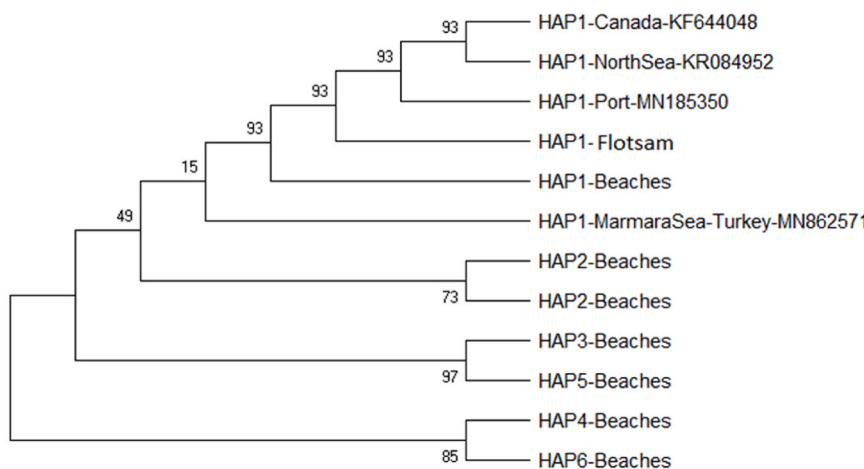


Fig. 5. A: Median-joining network showing the relationships among the *Magallana gigas* haplotypes defined by COI sequence variation. H\_1-H\_6 are the haplotype names. Areas in circles are proportional to the frequency of each haplotype. Color codes as blue, ports; yellow, beaches; black, flotsam. B: Maximum Parsimony analysis of haplotypes from *Magallana gigas*. Tree #1 out of 10 most parsimonious trees (length = 122) is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Sequences from Canada, the North Sea and Marmara Sea were included from NCBI databases (Accession numbers are provided). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

taxonomic composition of the three environments analyzed, thus confirming Hypothesis ii) (only a fraction of biopollutants that prefer plastic substrate will be transferred from port to beaches). In fact, the plastic flotsam here analyzed looks like a partially separated world, enriched in species belonging to the classes Malacostraca and Hexanauplia that make up a community that strongly differs from those observed in the port or on the beaches. These results are consistent with previous studies that indicate that marine litter will be fouled by a fraction of the total organisms inhabiting an environment, which will depend on a series of abiotic and biotic factors (Gibson et al., 2006). These factors include the ecological characteristics of the encrusting biota, such as having a long larval cycle, or being suspension feeders, which will be helpful to survive in abiotic substrates like marine litter where organisms depend on food resources from the surrounding environment (Kiessling et al., 2015). In the same way, the characteristics of marine litter (material, size, roughness) can also limit biofouling (Bravo et al., 2011), with large

plastic fragments generally allowing the adherence of a great diversity of species (Goldstein et al., 2014; Shabani et al., 2019).

This study also revealed similar density of shared and exclusive species in all the environment components, indicating that species shared are not denser in any environment than exclusive species. In other words, there are still exclusive species but many are shared regardless of the type of environment. Likewise, NIS and invasive species were not more frequently shared between environments than native species. This can be interpreted as artificial substrates in ports and flotsam are habitats (and vectors in the case of flotsam) of both NIS and native species in this region, as shown in the Baltic Sea (Garcia-Vazquez et al., 2018). The invasive condition would not determine the likelihood of occupation of artificial litter, which would act as a generic way of transport of any species (native or NIS) able to attach to it.

The high proportion of NIS found in the port (28.8% of the species) was consistent with previous results in the same area (Miralles et al.,

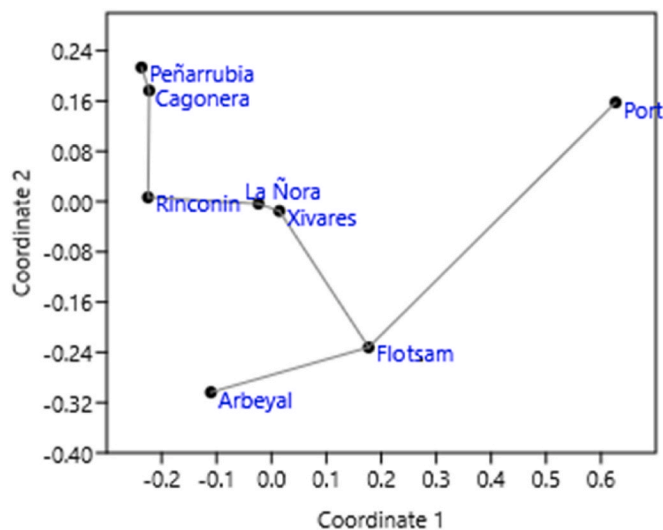


Fig. 6. Scatter plot of NMDS with minimum spanning tree, constructed from presence-absence of the species found in this study using Bray-Curtis distance and 9999 permutations.

2016), and also with results in very distant and ecologically different regions like Polynesia (Ardura et al., 2021). As expected, the port of Gijón was the environment component where more NIS were detected. This is due to the fact that ports are hotspots for the introduction of exotic species because of the intense maritime traffic, which is considered one of the most important pathways for the dispersal of marine species (López-Legentil et al., 2015; Ulman et al., 2017; Orlando-Bonaca et al., 2021). On the other hand, although having a lower proportion of NIS (22.2%), flotsam showed the highest densities of biopollutants, even surpassing the densities in the port surfaces. While all the NIS that were found on the beaches were also present in the port or in flotsam, the species attached to floating litter were shared in a great proportion with both natural and port areas and few of them were exclusive to flotsam. These results show the potential of the port of Gijón as a donor of NIS to the adjacent areas and remark the role of marine litter as a facilitator of their dispersion (Miralles et al., 2018a). Marine litter can be used by invasive species as a vector for the colonization of new areas. Clear examples in our study are the invasive species *Balanus trigonus* and *Magallana gigas* which were found alive fouling different litter items floating adrift on the coast near the port of Gijón. Moreover, the same COI haplotype was found in the port, flotsam and some nearby beaches for *Magallana gigas*, and in the port and flotsam for *Balanus trigonus*, reinforcing the idea of flotsam as a vector of invasive species between the port and other areas (Miralles et al., 2018a).

In our results, the beaches and beached objects seem to harbor different communities from those present in the flotsam. Interestingly, only a few beached objects had visible attached biota, while a large proportion of flotsam objects carried living macroscopic animals. Specimens might have been removed from the beached litter due to scavengers, desiccation, etc. The fauna associated to flotsam is still alive while the one on the beached objects might have been there but then washed away. It is worth mentioning that Arbeyal beach was not connected to the rest of the beaches in the NMDS (Fig. 5). This may be due to the influence of marine litter and NIS that may come from the marinas located near this beach. Previous studies have shown that the communities that inhabit marinas are slightly different from those found in ports (Miralles et al., 2021) and that non-industrial traffic from these marinas can also be a vector for the expansion of NIS to new areas. Marinas should also be considered when carrying out studies on the pathways of propagation that biopollutant species may employ for their dispersal.

The introduction and uncontrolled proliferation of exotic species can

have serious environmental consequences in the ecosystems which include competition for resources with native species, predation or the transmission of novel pathogens among others (Vilcinskas, 2015; Miaud et al., 2016; David et al., 2017; Yu et al., 2018). In this context, biological invasions can encompass threats to the sustainability of fishing resources and therefore, compromise the Sustainable Development Goal #14. Both marine litter and all the species found in this study should be taken into account for control and management to meet Sustainable Development Goal #14 in the Bay of Biscay. In order to avoid further deterioration of marine ecosystems and deal with biological invasions, there is an urgent need to reduce those pathways used by invasive species to disperse and enter new habitats, including marine litter. Among different actions that have been developed to reduce the presence of marine litter in aquatic ecosystems, the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) establishes a framework for the protection and sustainable use of marine ecosystems. This regulation also contemplates marine litter under the descriptor #10 that contains different considerations and proposals discussing the effectiveness of measures leading to reductions in marine litter (Galvani et al., 2013).

At a more local scale, campaigns such as “*Mójate por un mar sin residuos*” (Get involved in a sea without waste) or “*Basura a mares*” (Litter everywhere) have been carried out in Asturias, with the objectives of removing litter from the sea by fishermen or raising awareness among citizens about the problem posed by the litter that reaches the marine environment (Autoridad Portuaria de Gijón, 2018). In fact, to face the problem of marine litter, it is also necessary to generate citizen awareness to give rise to a more cooperative and involved society (Locritani et al., 2019). The reduction of marine litter will not only solve the problems that it causes to biota (intoxications, entanglements), but also prevent it from being used as a vector for the expansion of biopollutant species.

#### CRediT authorship contribution statement

**Sara Fernandez:** Data curation, Writing – review & editing. **Aitor Ibabe:** Writing – review & editing, Investigation. **Fernando Rayon-Viña:** Investigation, Writing – review & editing. **Alba Ardura:** Writing – review & editing. **Marlene Bartolomé:** Investigation, Writing – review & editing. **Yaisel J. Borrell:** Writing – review & editing, Validation. **Eduardo Dopico:** Investigation, Writing – review & editing. **Mónica Gonzalez:** Writing – review & editing, Resources. **Laura Miralles:** Resources, Writing – review & editing. **Horacio Montes:** Writing – review & editing, Investigation. **Trinidad Pérez:** Writing – review & editing. **Noemi Rodriguez:** Writing – review & editing. **Eva Garcia-Vazquez:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Conceptualization.

#### 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.

#### Acknowledgments

This study has been funded from the Spanish Ministry of Economy and Competitiveness Grant BLUEPORTS CGL-2016-79209-R and the Ministry of Science and Innovation Grant PDC2021-120939-I00. Sara Fernandez holds a Margarita Salas Grant with reference AYUD/2021/58385 We are grateful to three anonymous reviewers that helped to improve the manuscript very much. The authors want to honour the memory of our respected, loved co-author and friend Dr. Horacio Montes who passed away on April 2022.



## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2022.107879>.

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