Universidad de Oviedo
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University of Oviedo

Departamento de Biología Funcional

Programa de Doctorado: Programa Oficial de Doctorado en Ingeniería Química, Ambiental y Bioalimentaria

Tesis Doctoral

Marine plastic pollution as a vector for non-native species transport

Los plásticos contaminantes marinos como vector de transporte para especies exóticas

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RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

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RESUMEN (en español)

La contaminación y la introducción de especies no nativas (NIS) se encuentran entre las principales amenazas para la diversidad biológica global. Se ha demostrado que los plásticos flotantes son responsables de alteraciones significativas para el ser humano y para el medio ambiente. Entre los variados efectos negativos encontrados se encuentra su capacidad para transportar biota adherida, entre ellos NIS, a grandes distancias. Este fenómeno, llamado *rafting*, ha atraído recientemente la atención científica y pública, pero aún no existe una comprensión general del proceso, ni de su impacto a nivel global. El objetivo de esta tesis ha sido, por lo tanto, caracterizar de forma más exhaustiva el *rafting* en basura marina con origen antropogénico.

En este trabajo se han identificado, en primer lugar, lagunas de conocimiento y necesidades urgentes en la investigación sobre el *rafting* para evaluar su magnitud como vector en la introducción de NIS, y en su caso diseñar medidas para prevenirlo. La identificación de áreas de origen y receptoras, la clasificación de riesgo para los objetos flotantes y la contribución relativa de los desechos antropogénicos marinos a las invasiones biológicas globales se encuentran en un estado incipiente de

investigación a pesar de ser esenciales para diseñar estrategias de gestión eficientes.

Los estudios realizados en este trabajo con desechos antropogénicos y biota adherida demuestran que el *rafting* no es una excepción, sino que está presente en todas las zonas estudiadas. Se reportan aquí doce especies animales que nunca habían sido citadas sobre desechos antropogénicos flotantes. Un tercio de todas las especies identificadas fueron NIS. Los plásticos han sido identificados como el principal material de los objetos flotantes que transportan especies (*rafts*). Además, la composición taxonómica de la fauna adherida difiere entre *rafts* de diferentes materiales. Los plásticos no espumados transportan una fauna mucho más diversa que las espumas y los *rafts* no plásticos. Se ha podido demostrar incluso que la frecuencia de un taxón específico de la biota de *rafting* en una zona costera puede predecirse a partir del perfil de biota característico de cada material y la composición de desechos antropogénicos en las playas.

Las áreas de acuicultura se han identificado en esta tesis como zonas de alto riesgo como donantes de *rafting* de NIS. La mayoría de *rafts* y los NIS adheridos a ellos que se encontraron en muestras de dos zonas costeras diferentes (Portugal, Atlántico e Italia, Mediterráneo) estaban directamente relacionados con las actividades acuícolas. Es esencial, por tanto, prevenir las pérdidas de material derivadas de las actividades humanas en el mar, en particular la acuicultura. Más aún, hay que evitar que la basura antropogénica de cualquier origen se incorpore al medio marino; en este estudio se detectó también una alta frecuencia de fragmentos y objetos no identificados entre los *rafts*, cuya fuente no pudo determinarse.

El *rafting* de especies invasoras es más común en el Atlántico Norte y el Mediterráneo que en el entorno del Pacífico Sureste. Sin embargo, en este estudio se ha demostrado que la remota Rapa Nui (Isla de Pascua) recibe grandes cantidades de desechos flotantes con biota adherida procedente del Giro Subtropical del Pacífico Sur. Probablemente debido a la ocurrencia mucho más baja de NIS a lo largo de costas del Pacífico sureste, entre las especies no nativas encontradas no se hallaron especies invasoras en los *rafts* de la isla. Esto sugiere que la introducción o dispersión de NIS mediante el *rafting* en la basura marina antropogénica es un riesgo principalmente en las áreas oceánicas donde los NIS ya han sido introducidos por otros vectores, como pueden ser el tráfico marítimo o la acuicultura.

En resumen, los resultados obtenidos en esta tesis demuestran que el rafting en la basura antropogénica es un fenómeno con una dimensión global. Sin embargo, la importancia y el impacto de esta forma de transporte para los NIS parece depender de la región geográfica y de si previamente ya se habían introducido en el área en cuestión debido a otros vectores. Este trabajo ha detectado muchas especies (incluyendo NIS) ya conocidas como típicas en desechos antropogénicos flotantes. Además de las especies habituales, en cada zona muestreada, incluyendo las estudiadas en esta tesis, se revelan nuevas especies y nuevos NIS haciendo rafting. En un escenario de cambio climático acelerado, con recurrentes fenómenos de alteraciones ambientales, son necesarias nuevas leyes, políticas y campañas efectivas de concienciación ciudadana y empresarial para reducir de forma significativa la basura antropogénica que se vierte al mar.

RESUMEN (en Inglés)

Invasive species and pollution are among the main threats to global species diversity. Floating or stranded plastics have many negative effects on the environment and human beings, amongst them the ability of plastics to transport attached biota, including non-native and/or invasive species, over large distances. This phenomenon, called rafting, is recently gaining scientific and public attention, but there is not yet an overall understanding of the process and its global impact. The aim of this thesis was therefore to gain comprehensive understanding of biota rafting on anthropogenic marine litter.

As a result of this PhD thesis, main knowledge gaps have been identified and addressed. Several research needs for evaluating and preventing the imminent, biodiversity-threatening problem of non-native invasive species (NIS) carried by anthropogenic marine litter have been identified. Donor and vulnerable recipient areas, high risk litter items, and the relative contribution of marine litter to global biological invasions are main issues that need to be addressed to design efficient management strategies.

Stranded anthropogenic litter items with attached biota were found on almost every beach sampled, showing that rafting is not an exception, but ubiquitous. Moreover, twelve species were found, which had never been reported rafting on anthropogenic

marine litter before. One third of all identified rafting species were NIS. Plastics have been identified as the main vector material. The taxon composition of the attached rafting fauna differed between rafts of different materials, with non-foamed plastics carrying a much more diverse rafting fauna than foams and non-plastics rafts. It was found that the frequency of a specific taxon of rafting biota in a coastal area may be predicted based on each litter material's characteristic biota profile and the beaches' litter composition.

Areas with high levels of activity in the aquaculture sector have been identified in this thesis as high-risk areas of origin for NIS rafting. The majority of rafts and adhered NIS found in samplings in two different coastal zones (Algarve, Atlantic and Venice, Mediterranean) were directly related to aquaculture activities. This shows that the prevention of losses from sea-based activities, particularly aquaculture, is important for the prevention of rafting of non-native invasive species. However, the high frequency of unidentified fragments and objects among rafts, whose source cannot be identified, also shows the importance of preventing anthropogenic litter from entering the marine environment in the first place.

It has been shown that NIS rafting is more common in the North Atlantic and the Mediterranean than in the Southeast Pacific environment. In this study it has also been shown that the remote Rapa Nui (Easter Island) receives large amounts of floating debris with adhered biota from the South Pacific Subtropical Gyre. However, probably due to the much lower occurrence of NIS along the southeastern Pacific coast, no invasive species were found among the non-native species on rafts on the island. This suggests that the introduction or dispersion of NIS through rafting in anthropogenic marine litter is mainly a risk in oceanic areas where NIS have already been introduced by other vectors, such as marine traffic or aquaculture.

In summary, the results obtained in this thesis show that rafting on anthropogenic marine litter is a common global phenomenon. However, the importance and impact of this form of NIS transport seems to depend on the geographic region and the presence of previously introduced NIS. This work has detected many species (including NIS) already known as typical in floating anthropogenic litter. However, every study conducted (including those included in this Thesis) reveals new species (and new NIS) by rafting. In a scenario of accelerated climate change and recurrent environmental phenomena, new laws, policies and effective citizen and industrial awareness campaigns are needed to significantly reduce the anthropogenic waste that is dumped into the sea. The success of this approach can help to prevent and better manage the global phenomenon of biological invasions.

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To the team of AZTI tecnalia in Basque Country and the amazing colleagues of the Swansea University Biosciences department for a great time!

Tengo los lagos, tengo los ríos
Tengo mis dientes pa` cuando me sonrío
La nieve que maquilla mis montañas
Tengo el sol que me seca y la lluvia que me baña
Un desierto embriagado con bellos de un trago de pulque
Para cantar con los coyotes, todo lo que necesito
Tengo mis pulmones respirando azul clarito
La altura que sofoca
Soy las muelas de mi boca mascando coca
El otoño con sus hojas desmalladas
Los versos escritos bajo la noche estrellada
Una viña repleta de uvas
Un cañaveral bajo el sol en cuba
Soy el mar caribe que vigila las casitas
Haciendo rituales de agua bendita
El viento que peina mi cabello
Soy todos los santos que cuelgan de mi cuello
El jugo de mi lucha no es artificial
Porque el abono de mi tierra es natural

Tú no puedes comprar al viento, Tú no puedes comprar al sol
Tú no puedes comprar la lluvia, Tú no puedes comprar el calor
Tú no puedes comprar las nubes, Tú no puedes comprar los colores
Tú no puedes comprar mi alegría, Tú no puedes comprar mis dolores

(Calle 13, América Latina)


NO TE PEDIMOS QUE LIMPIES LA PLAYA,
SOLO TE PEDIMOS
QUE NO LA ENSUCIES.
HAZTE CARGO DE TU BASURA
ILUSTRE MUNICIPALIDAD DE ARICA
CORPESCA
PIENSA
LO QUE BOTAS NO DESAPARECE
TODO TIENE UN
TIEMPO DE DEGRADACION
PAPEL ....DE 2 A 5 MESES
CIGARRO....DE 1 A 12 AÑOS
CARTON....5 AÑOS
CHICLE....5 AÑOS
LATAS....DE 80 A 100 AÑOS
PLASTICOS....DE 100 A 1000 AÑOS
VIDRIO....DE 1000 A 4000 AÑOS
HAZTE CARGO DE TU BASURA


Cuidemos el Mar

Photos: above: Arica, Chile. Below: Coquimbo, Chile. Photos by Sabine Rech

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RESUMEN

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difiere entre *rafts* de diferentes materiales. Los plásticos no espumados transportan una fauna mucho más diversa que las espumas y los *rafts* no plásticos. Se ha podido demostrar incluso que la frecuencia de un taxón específico de la biota de *rafting* en una zona costera puede predecirse a partir del perfil de biota característico de cada material y la composición de desechos antropogénicos en las playas.

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En resumen, los resultados obtenidos en esta Tesis demuestran que el rafting en la basura antropogénica es un fenómeno con una dimensión global. Sin embargo, la importancia y el impacto de esta forma de transporte para los NIS parece depender de la región geográfica y de si previamente ya se habían introducido en el área en cuestión debido a otros vectores. Este trabajo ha detectado muchas especies (incluyendo NIS) ya conocidas como típicas en desechos antropogénicos flotantes. Además de las especies habituales, en cada zona muestreada, incluyendo las estudiadas en esta Tesis, se revelan nuevas especies y nuevos NIS haciendo rafting. En un escenario de cambio climático acelerado, con recurrentes fenómenos de alteraciones ambientales, son necesarias nuevas leyes, políticas y campañas efectivas de concienciación ciudadana y empresarial para reducir de forma significativa la basura antropogénica que se vierte al mar.

SUMMARY

Invasive species and pollution are among the main threats to global species diversity. Floating or stranded plastics have many negative effects on the environment and human beings, amongst them the ability of plastics to transport attached biota, including non-native and/or invasive species, over large distances. This phenomenon, called rafting, is recently gaining scientific and public attention, but there is not yet an overall understanding of the process and its global impact. The aim of this Thesis was therefore to gain comprehensive understanding of biota rafting on anthropogenic marine litter.

As a result of this PhD Thesis, main knowledge gaps have been identified and addressed. Several research needs for evaluating and preventing the imminent, biodiversity-threatening problem of non-native invasive species (NIS) carried by anthropogenic marine litter have been identified. Donor and vulnerable recipient areas, high risk litter items, and the relative contribution of marine litter to global biological invasions are main issues that need to be addressed to design efficient management strategies.

Stranded anthropogenic litter items with attached biota were found on almost every beach sampled, showing that rafting is not an exception, but ubiquitous. Moreover, twelve species were found, which had never been reported rafting on anthropogenic marine litter before. One third of all identified rafting species were NIS. Plastics have been identified as the main vector material. The taxon composition of the attached rafting fauna differed between rafts of different materials, with non-foamed plastics carrying a much more diverse rafting fauna than foams and non-plastics rafts. It was found that the frequency of a specific taxon of rafting biota in a coastal area may be predicted based on each litter material's characteristic biota profile and the beaches' litter composition.

Areas with high levels of activity in the aquaculture sector have been identified in this Thesis as high-risk areas of origin for NIS rafting. The majority of rafts and adhered NIS found in samplings in two different coastal zones (Algarve, Atlantic and Venice, Mediterranean) were directly related to aquaculture activities. This shows that the prevention of losses from sea-based activities, particularly aquaculture, is important for the prevention of rafting of non-native invasive species. However, the high frequency of unidentified fragments and objects among rafts, whose source cannot be identified, also shows the importance of preventing anthropogenic litter from entering the marine environment in the first place.

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In summary, the results obtained in this Thesis show that rafting on anthropogenic marine litter is a common global phenomenon. However, the importance and impact of this form of NIS transport seems to depend on the geographic region and the presence of previously introduced NIS. This work has detected many species (including NIS) already known as typical in floating anthropogenic litter. However, every study conducted (including those included in this Thesis) reveals new species (and new NIS) by rafting. In a scenario of accelerated climate change and recurrent environmental phenomena, new laws, policies and effective citizen and industrial awareness campaigns are needed to significantly reduce the anthropogenic waste that is dumped into the sea. The success of this approach can help to prevent and better manage the global phenomenon of biological invasions.

Introduction

INTRODUCTION

Invasive species and diseases, as well as pollution are among the five main threats to global species diversity (Joppa et al., 2016; Maxwell et al., 2016). This includes the marine environment, where a major pollutant is litter from anthropogenic sources, particularly plastics (Rochman et al., 2013; Haward, 2018). Both problems, invasive species and marine plastic pollution, have gained scientific and public attention during the last decades, with several programs having been initiated to investigate and ideally solve these problems (e.g. Bax et al., 2003; Eastman et al., 2014; Tricarico et al., 2017; Haward, 2018). The present Thesis investigates the connection between those two major environmental threats, particularly the role of marine plastic pollution in transporting non-native, invasive species.

1. Plastic pollution

1.1. The plastic problem – an overview

Plastics were first introduced to the wide public after the second world war. While they were first advertised and appreciated as a material that would make people´s daily lives easier and more comfortable (Figure 1), today they are a major threat to the environment and difficult to avoid in everyday live in most consumerist societies (e. g. food packaging, Figure 2).

Figure 1. Advertisement for plastic foil. Source: The advertising archives; www.dailymail.co.uk

Figure 2. Peeled oranges in plastic packaging. Photo: Nathalie Gordon.

Their characteristic traits - light weight, durability, and cheapness - make them the preferred material for everyday items, but at the same time make them a persistent environmental polluter (Vegter et al., 2014). The problems posed by plastic pollution are now widely acknowledged by the public, media, governments, and NGOs, but production is still increasing every year (Fernandes and Sansolo, 2013; Löhr et al., 2017). In 2016, global plastic production reached a level of 335 million tonnes – 13 million tonnes more than in 2015 (Plastics Europe, 2018). Several campaigns are being conducted to combat the excessive production and consumption of single-use plastics, for example plastic bags from supermarkets, microbeads in cosmetic products, or PET (Polyethylene terephthalate) beverage bottles (e.g. http://storyofstuff.org/, http://www.beatthemicrobead.org/). Policy changes have been requested after increasing scientific evidence and public awareness about the pollution problem (Clapp and Swanston, 2009; Doughty and Eriksen, 2015). While possible measures to mitigate the problem have often focused on consumer behaviour and have largely failed, extended producer responsibility (EPR) is suggested to tackle the problem at its root, holding industry responsible (Eriksen, 2014). In spite of the efforts made by many organisations and individuals, the *„ultimately … entirely avoidable problem"* (Vegter et al., 2014) of plastic pollution is far from being solved – with detrimental impacts on nature and human interests, which are particularly grave in the marine environment.

1.2. Plastics in the marine environment

Nowadays no known part of the marine environment is free of plastics. The impacts of floating or stranded marine litter are manifold, including injuries and death of marine biota caused by ingestion of plastics and entanglement in litter items, like plastic bags or fishing nets or the spreading of invasive species (Kiessling et al., 2015; Fossi et al., 2018; Thiel et al., 2018; Figure 3).

Figure 3. Marine turtle entangled in derelict fishing gear. Photo: Jordi Chias. Source: https://oceanchampions.ca/

Sinking litter forms artificial hardgrounds, thereby changing sediment composition and posing a potential threat to the structure of benthic communities (Moore, 2008; Gregory, 2009). Similarly, Carson et al. (2011) found that small plastic litter changes the characteristics of beach sediment with respect to water movement and heat transfer, with unknown consequences for the organisms of this habitat. Marine litter also has direct negative effects on human interests, especially with regard to coastal activities and industries, like shipping, fishing or tourism, by damaging vessel machinery and posing difficulties to navigation (Macfadyen et al., 2009; McIlgorm et al., 2011). When washing up on beaches, anthropogenic litter reduces their aesthetic value and poses health risks to beach-goers, which reduces the touristic attraction of the affected area (Sheavly and Register, 2007; Williams et al., 2016). The effects of anthropogenic litter are not only detrimental to the marine environment, but also to human economies. A recent report of the United Nations Environment Program (UNEP) estimates the total natural capital cost of marine plastic pollution to be at least US$13 billion (UNEP, 2014). Last but not least, plastics in the marine environment pose a dangerous threat to human health, as they not only contain, but moreover adsorb hazardous chemicals that can enter animals' tissues when ingested and can be transferred along the food chain (Rochman et al., 2013; Nelms et al., 2018). While most of the above-mentioned aspects have been investigated thoroughly, the transport of (non-native) biota by floating anthropogenic objects is a phenomenon that has only attracted scientific and public attention in the last years and its extent and impact are not well known yet. The present Thesis therefore aims to give insight in the processes and overall impact of rafting on anthropogenic marine litter.

2. Floating anthropogenic litter as a transport vector

2.1. Rafting – an overview

Rafting is the process of biota being transported by floating material in the marine environment. It is by no means a new phenomenon - dispersal of marine and coastal organisms on floating material, such as macroalgae, volcanic pumice, or drift wood is well known and has importantly influenced biotic communities on islands (Thiel and Gutow, 2005a; Fraser et al., 2011; Nikula et al., 2013; Kiessling et al., 2015). The phenomenon of rafting on natural substrates has been intensively reviewed (Thiel and Gutow, 2005a, 2005b; Thiel and Haye, 2006). Although Carpenter and Smith (1972) already suggested that plastics may importantly increase settlement options for biota in the marine environment, little research was conducted on the topic. 30 years later, Barnes (2002) suggested that marine plastic litter may actually more than double rafting opportunities. There are now several reports on major rafting events, as well as many anecdotal reports of rafting on anthropogenic litter, but the extend and impact of the phenomenon remain unclear.

Floating litter is a vector for both first introductions (long distance transport) in a new region, and secondary spread of NIS (short-distance transport) within an already affected region. However, as in much of the available literature rafting is usually referred to as "other routes of introduction" (Katsanevakis and Crocetta, 2014), the actual contribution of floating litter to the introduction and spreading of NIS remains unknown (Vegter et al., 2014). In the Mediterranean, for example, rafting is suggested to be a potentially important vector of both primary NIS introductions via corridors, as well as of secondary spread of already introduced species (Katsanevakis and Crocetta, 2014). With more than 80% of alien species in the Mediterranean possibly having arrived on floating litter or used this vector for further dispersal, its importance might be seriously underestimated (Galgani et al., 2014). In British brackish and marine waters, floating litter is the third most common vector of alien species introductions (Minchin et al., 2013). There are many examples of long and medium-distance transport of biota along the prevailing oceanic currents in different regions (Thiel and Haye, 2006; Gregory, 2009; Kiessling et al., 2015). A recent massive rafting event was the transport of 289 living marine coastal species on Japanese tsunami marine debris (JTMD) objects, that stimulated research and gives ongoing insight in many rafting-related issues (Calder et al., 2014; Carlton et al., 2017; McCuller and Carlton, 2018; Miller et al., 2018). JTMD objects, like fishing vessels, large docks and buoys, amongst others, were detached off Japan by a tsunami, caused by the 2011 Japan earthquake, and travelled across the Pacific Ocean over several years and thousands of kilometres, carrying a variety of attached biota in large densities (Carlton et al., 2017; Figure 4).

Figure 4. Japanese tsunami marine debris (JTMD) objects, carrying attached biota. Source: Carlton et al., 2017.

A recent trans-Atlantic rafting event was reported from British and Irish shores in winter of 2013-2014. Following prolonged westerly gales, large numbers of rafting biota, including non-indigenous molluscs and barnacles, attached to plastic litter of North American origin, washed up on local beaches (Holmes et al., 2015). Here again, the majority of biota was still alive when found and/or showed other signs of long-term survival (Holmes et al., 2015). The importance of marine litter for near-shore NIS dispersal, where the first introduction occurred due to another vector (secondary spread) has also been emphasized by several authors (e.g. Winston et al., 1997). The relative frequency of each type of transport (long- or short- distance), and especially the contribution of litter on regional NIS spread remains to be quantified.

2.2. Rafting biota and transport of non-native invasive species

In their 2015 review, Kiessling et al. (2015) list 387 taxa that have been found rafting or attached to stranded litter of anthropogenic origin. The most frequent groups found are sessile invertebrates, particularly bryozoans, crustaceans, molluscs and cnidarians, although it is suggested that this finding may be due to the prevalence of beach samplings (in contrast to sampling of floating litter), where mobile organism might already have left their raft when it is found (Kiessling et al., 2015). Mobile crustaceans and annelids, for example are more frequent in studies on floating rafts (Astudillo et al. 2009; Goldstein et al. 2014). In general, most of the rafting organisms found in previous reports are suspension feeders and have an extended larval development. Many of the species colonizing anthropogenic marine litter items are common or even obligate rafters, such as goose barnacles of the genera *Lepas* and *Dosima* (Kiessling et al., 2015; Figure 5).

Figure 5. Lepas sp. on buoys stranded on a beach. Photo: Sabine Rech.

A major concern is the transport of non-native invasive species (NIS) by floating anthropogenic marine litter. Many of the species found rafting in the marine environment are invasive outside of their native range (Kiessling et al., 2015). Most of the species transported by Japanese tsunami marine litter to North American shores were non-native to this region and several of them were classified as high-risk invaders, due to their previous invasion history (Figure 6). The species with the highest invasion risk were the mussel *Mytilus galloprovincialis,* the ascidian *Didemnum vexillum*, and the crab *Hemigrapsus sanguineus* (Therriault et al., 2018). Similarly, the transatlantic rafting event, reported by Holmes et al. (2015), brought several non-native and possibly invasive mollusc species to British and Irish shores. Apart from long-distance dispersal, rafting on marine litter has been suggested to be involved in regional dispersal of several invertebrates (Lutz-Collins et al., 2009; Whitehead et al., 2011; Serrano et al., 2013). For example, juveniles of the bivalve *Pinctada imbricata* and adults of *Isognomon bicolor*, which are considered invasive in Brazil, were found attached to anthropogenic litter for the first time at the Uruguayan coast, where they are regarded as potentially invasive as well (Breves et al., 2014; Marques and Breves, 2015). In the Spanish part of the Bay of Biscay, several non-native invasive species are registered (Ministerio de Agricultura Alimentación y Medio Ambiente, 2013), some of which are already known to attach to floating anthropogenic marine litter in other regions (Kiessling et al., 2015). Examples are the pygmy mussel *Xenostrobus securis* as well as the oysters *Crassostrea gigas* and *Ostrea stentina* (Adarraga and Martínez, 2012; Pejovic et al., 2016). According to EU Regulation No 1143/2014 there are about 12,000 alien species in European countries, of which 10–15% are regarded as invasive and pose a serious threat to the environment and human interests (Regulation (EU) No 1143/2014, 2014).

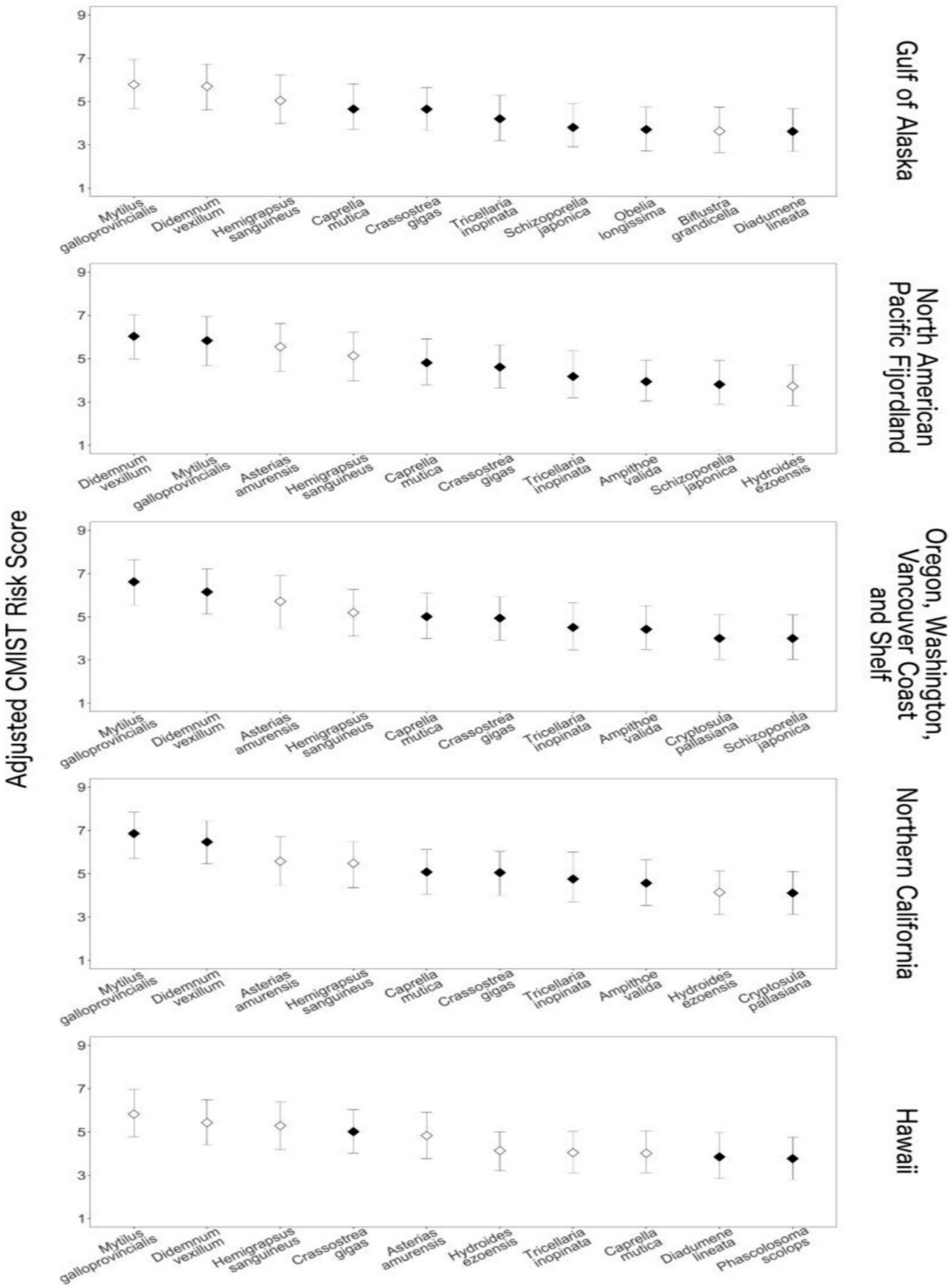

Figure 6. The ten highest risk invertebrate species from Japanese tsunami marine debris by ecoregion: Gulf of Alaska, North American Pacific Fijordland, Oregon, Washington, Vancouver Coast and Shelf, Northern California, and Hawaii. From: Therriault et al., 2018.

Although there are now numerous reports of non-native invasive biota rafting on anthropogenic marine litter, it remains to be estimated how important this form of transport is in comparison to other vectors, like the accidental transport on ship hulls or in ballast water, or the introduction by aquaculture. Moreover, the arrival of non-native invasive biota is not necessarily followed by a successful colonization of the new habitat. To determine the proportion of non-native biota arriving via rafting on floating litter, that establish colonies in the new habitat will require substantial future sampling effort. However, the fact that rafting has been detected as the most likely vector of introduction for at least 9% of alien species in the British isles, and may have been a vector for a large part of alien introductions in the Mediterranean shows that research on the topic is well-invested (Minchin et al., 2013; Katsanevakis and Crocetta, 2014).

2.3. Sources, pathways and sinks of rafts and rafting biota

2.3.1. Source areas and activities

It is estimated that about 80% of anthropogenic litter in the marine environment stems from land-based sources (Andrady, 2011). These can be from the coastal environment, like coast-based industry or beach tourism, or from the inland, where anthropogenic litter enters rivers and waterways from households, industries, or refuse sites and is then transported to the sea (Rech et al., 2014; Lebreton et al., 2017). Waste water treatment is insufficient in many global regions, one of them being the Asturian (Spain) coast, where most of the samplings in this Thesis were conducted (European Comission, 2018). Where water treatment systems are available, they mostly retain larger litter items, but not microplastics, like industrial preproduction pellets, getting lost during manufacture and transport, particles from cosmetics and facial cleansers, or microfibers from synthetic clothing, which enter the waterways via domestic or industrial drainage systems (Mato et al., 2001; Andrady, 2011; Cole et al., 2011). Sea-based activities like vessel-traffic, fishery, or off-shore oil industry also introduce anthropogenic litter into the sea, due to either losses or irregular disposal (Andrady, 2011; Cole et al., 2011). Several legislation efforts have been made to reduce littering from vessels, but compliance and enforcement remain problematic (Bergmann et al., 2015). Some anthropogenic items, like buoys, are already fouled and carry attached biota when they get lost or become detached, while other items enter the marine environment unfouled and become colonized by biota later.

The identification of source areas is a priority for the prevention of litter input and subsequent rafting by NIS (Goldstein et al., 2014). High-risk areas are those where intense littering coincides with a high occurrence of potential invasive species. Ports and marinas, for example, offer suitable shelters for NIS and might become source sites for NIS rafting on floating anthropogenic marine litter, especially when located in densely populated zones with a high amount of litter (Ashton et al., 2006; Glasby et al., 2007; Tyrrell and Byers, 2007; Seebens et al., 2013; Peters et al., 2014; Wells et al., 2014; Ardura et al., 2015; Pejovic et al., 2016).

Aquaculture sites have been identified as (potential) donor areas for rafting invasive species by several authors (Hewitt et al., 2006; Cook et al., 2008; Arthur et al., 2009; Campbell et al., 2017). According to current research, aquaculture is the second most important pathway of marine alien species introduction to European seas, and a largely underestimated role of marine litter in such introductions is suggested (Katsanevakis et al., 2013; Katsanevakis and Crocetta, 2014). Aquaculture, often located in estuaries, is economically and ecologically affected by fouling organisms and plastic pollution (Williams and Grosholz, 2008; Rius et al., 2011; Sussarellu et al., 2016). At the same time it is a major source of NIS, due to escapes - and sometimes active releases - of exotic farmed individuals (Rius et al., 2011; Crego-Prieto et al., 2015; Habtemariam et al., 2015; Semeraro et al., 2015). The floating devices used in aquaculture often provide optimal conditions for fouling NIS, especially when they get detached (Rius et al., 2011; Katsanevakis et al., 2013; James and Shears, 2016). Considerable amounts of detached buoys with attached NIS, as well as floating litter from aquaculture activities was reported from some locations, especially related to extreme climatic events (Astudillo et al., 2009; Hinojosa and Thiel, 2009; Macfadyen et al., 2009; Liu et al., 2015).

2.3.2. Pathways

Having entered the marine environment, floating items and attached biota are moved with oceanic currents and accumulate in oceanic divergence zones. The largest and most important are the five oceanic gyres, situated in the North- and South Pacific, North-and South Atlantic and Indian Ocean (Law et al., 2010; Eriksen et al., 2013; Goldstein et al., 2013; Ryan, 2014; Figure 7).

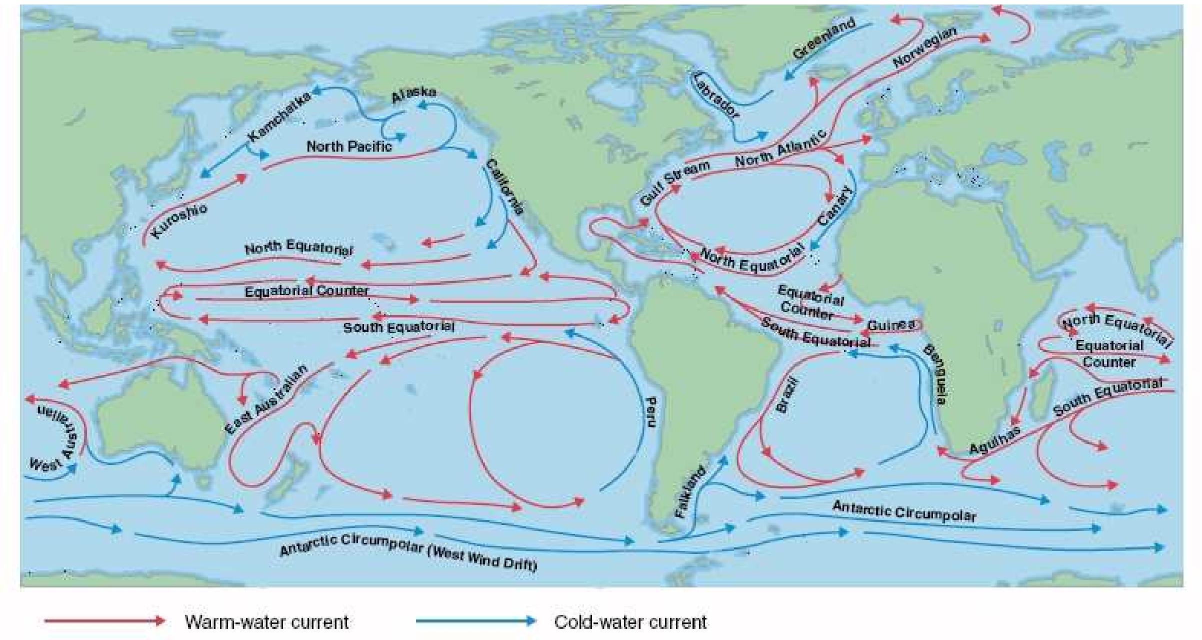

Figure 7. The oceanic gyres. Image: SSEC, University of Wisconsin-Madison. Source: https://cimss.ssec.wisc.edu/.

Contrary to widespread popular opinion, these accumulations are not visible litter islands, but are defined by significantly higher concentrations of litter items and particles compared to oceanic waters outside the gyres (Eriksen et al., 2013, 2014; Ryan, 2014; Figure 8).

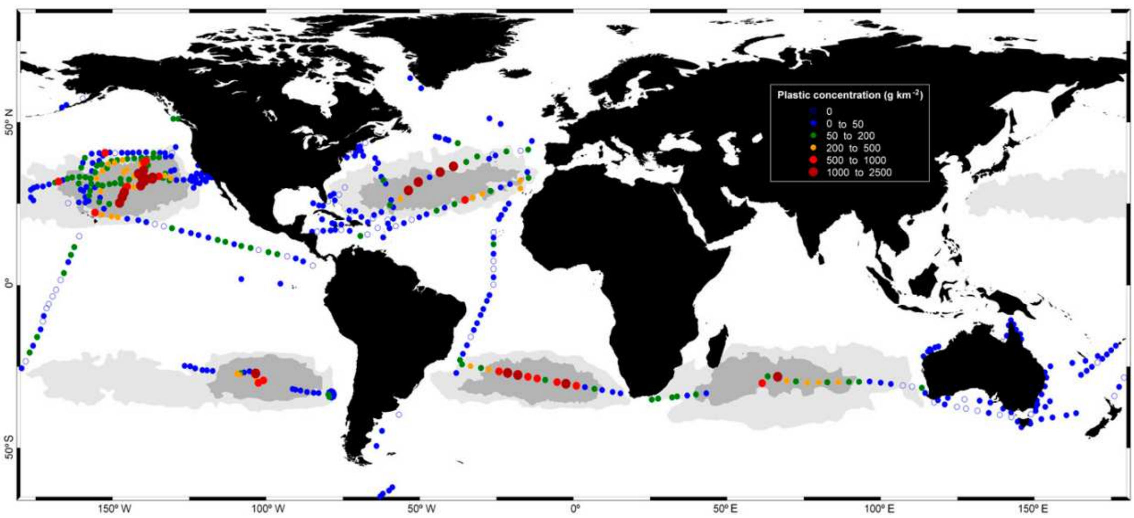

Figure 8. Concentrations of floating plastic litter in surface waters of the global oceans. Source: Cózar et al., 2014.

It is suggested that rafting biota may be travelling within these gyres for several years before reaching land (Hoeksema et al., 2012).

2.3.3. Sink areas

As marine litter is primarily dispersed by surface currents, driven by Ekman currents and local winds, all natural marine sink areas are sinks for floating litter as well. In concordance with the accumulation of floating litter in the oceanic gyres, high accumulations of stranded litter are often found in coastal areas and on oceanic islands in close proximity to the gyres (Hidalgo-Ruz and Thiel, 2013). For example, a significant rise of marine litter accumulation over the last 30 years has been documented from remote islands of the southern hemisphere, and it has been shown that South Pacific islands accumulate exceptionally high quantities of marine litter (Barnes, 2005; Lavers and Bond, 2017; Hidalgo-Ruz et al., 2018). Barnes and Milner (2005) found the globally highest accumulations of stranded litter in subtropical areas, which is in concordance with the location of the subtropical gyres, as well as on Mediterranean shores (Figure 9). Moreover, they found a significant decline in colonization of marine litter items with latitude: The share of colonized litter items fell from 50% at low latitudes (0 - 15°) to 25% at higher latitudes (15 – 40°) and decreased further towards the poles (Barnes and Milner, 2005; Kiessling et al., 2015; Figure 9). This emphasizes that the tropical and subtropical environments are in particular risk of receiving biota rafting on anthropogenic marine litter.

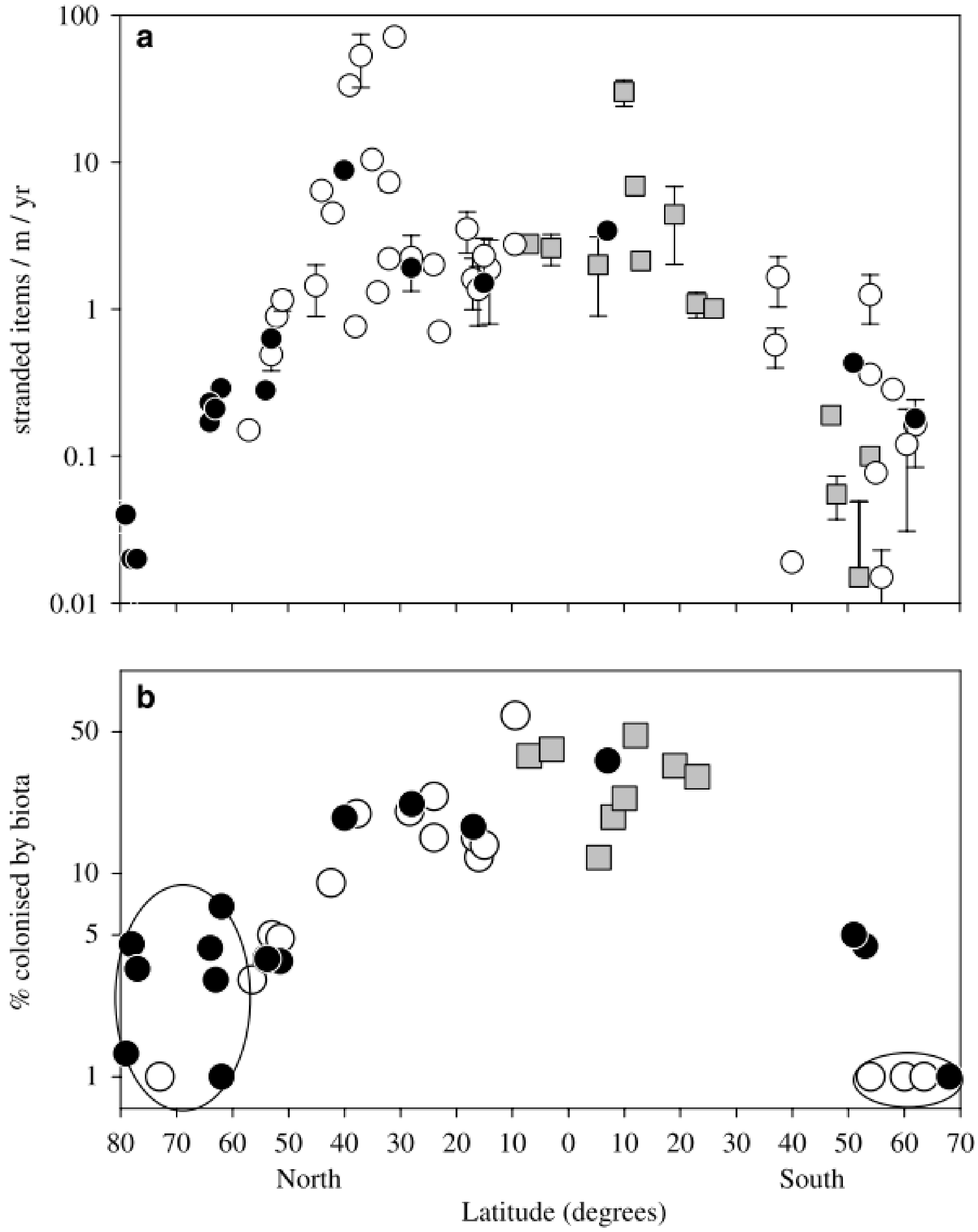

Figure 9. Abundance of stranded litter items and percentage of colonized items with latitude. Source: Barnes and Milner, 2005.

The deposition of marine litter in sink areas is often aggravated by storms (Doong et al., 2011; Lebreton and Borrero, 2013; Holmes et al., 2015). Litter accumulation along coastlines is mainly driven by near-shore currents and winds, tidal dynamics, wave motion and coastal geomorphology (Araújo and Costa, 2007a, 2007b; Browne et al., 2010; Doong et al., 2011; Carson et al., 2013; Critchell and Lambrechts, 2016). Figure 10 shows an example of a beach with a dense accumulation of stranded matter and anthropogenic marine litter.

Figure 10. Beach with dense accumulation of stranded litter. Photo: Sabine Rech.

3. Thesis aims

The aim of the present Thesis was to gain comprehensive understanding of the global phenomenon of biota rafting on marine anthropogenic litter items. Therefore, areas that represent several geographic regions and have high accumulations of floating litter were chosen for the samplings and investigations:

1) The Bay of Biscay in the Northern Atlantic has a variety of possible litter sources (aquaculture, fisheries, ports, coastal population and coastal tourism, industry) and is connected with the North Atlantic Gyre via the North Atlantic Drift. It was chosen as a sampling area to investigate high-risk and most common items transporting attached biota and to identify species/ taxon preferences for specific types of litter.

2) The Mediterranean Sea has high accumulations of anthropogenic marine litter and a high number of non-native invasive species (NIS). It comprises a large part of Europe´s aquaculture facilities. Together with the Portuguese Algarve region, which is another important region for shellfish culture and strongly affected by NIS, it was chosen to investigate the impact of aquaculture, as a suspected source of both non-indigenous species and marine litter, in the release of non-native rafting species and rafts of anthropogenic origin.

3) Rapa Nui (Easter island) is a remote island in the centre region of the South Pacific Subtropical Gyre, with a relatively small population. Despite its remoteness and the

scarceness of local litter sources, very high accumulations of anthropogenic marine litter have been reported from the island. It was therefore chosen to assess the impact of rafting on anthropogenic litter as a mechanism of species introduction and dispersal in remote areas, where other vectors of species introduction are absent or scarce.

Objectives

OBJECTIVES

The overall goal of this PhD Thesis was to create a comprehensive understanding of the subject of biota transport by marine anthropogenic litter on a global scale, helped by DNA barcoding for species identification. The specific objectives were:

1) To conduct a thorough literature search and summarize the existing knowledge and records of anthropogenic marine litter as a transport vector for attached biota.

2) To define high-risk and most common items transporting attached biota and identify species/ taxon preferences for specific types of litter, using DNA barcoding as a tool, to predict rafted biota based on the composition of stranded litter.

3) To investigate the impact of aquaculture, as a suspected source of both non-indigenous species and marine litter, in the release of non-native rafting species and rafts of anthropogenic origin.

4) To assess the impact of rafting on anthropogenic litter as a mechanism of species introduction and dispersal in remote areas, where other vectors of species introduction are absent or scarce.

Results

RESULTS

Chapter 1: Marine litter as a vector for non-native species: What we need to know

Publication: Rech S, Borrell YJ, Garcia-Vazquez E. (2016). Marine litter as a vector for non-native species: What we need to know. Marine Pollution Bulletin 113: 40-43

Chapter 2: Anthropogenic marine litter composition in coastal areas may be a predictor of potentially invasive rafting fauna

Publication: Rech S, Borrell YJ, Garcia-Vazquez E. (2018). Anthropogenic marine litter composition in coastal areas may be a predictor of potentially invasive rafting fauna. PLoS ONE 13(1): e0191859

Chapter 3: Dispersal of alien invasive species on anthropogenic litter from European mariculture areas

Publication: Rech S, Salmina S, Borrell YJ, Garcia-Vazquez E. (2018). Dispersal of alien invasive species on anthropogenic litter from European mariculture areas. Marine Pollution Bulletin 131: 10-16

Chapter 4: Travelling light: Fouling biota on macroplastics arriving on beaches of remote Rapa Nui (Easter Island) in the South Pacific Subtropical Gyre

Publication: Rech S, Thiel M, Borrell YJ, Garcia-Vazquez E. (2018). Travelling light: Fouling biota on macroplastics arriving on beaches of remote Rapa Nui (Easter Island) in the South Pacific Subtropical Gyre. Marine Pollution Bulletin 137: 119-128

CHAPTER 1

Marine litter as a vector for non-native species: What we need to know

Rech S, Borrell YJ, Garcia-Vazquez E.

Marine Pollution Bulletin 2016, 113: 40-43

Photo by Sabine Rech

Este capítulo (p. 42bis) se corresponde con el artículo:

Rech, S., Borrell, Y., & García-Vazquez, E. (2016). *Marine litter as a vector for non-native species: What we need to know*. En **Marine Pollution Bulletin**, 113 (1-2), p. 40–43 (2016); doi:10.1016/j.marpolbul.2016.08.032

Debido a la política de autoarchivo de la publicación la versión de la editorial está disponible, únicamente para usuarios con suscripción de pago a la revista, en el siguiente enlace: http://dx.doi.org/10.1016/j.marpolbul.2016.08.032

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CHAPTER 2

Anthropogenic marine litter composition in coastal areas may be a predictor of potentially invasive rafting fauna

Rech S, Borrell YJ, Garcia-Vazquez E.

PLoS ONE 2018, 13(1): e0191859

Photo by Sabine Rech

RESEARCH ARTICLE

Anthropogenic marine litter composition in coastal areas may be a predictor of potentially invasive rafting fauna

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Abstract

Anthropogenic plastic pollution is a global problem. In the marine environment, one of its less studied effects is the transport of attached biota, which might lead to introductions of non-native species in new areas or aid in habitat expansions of invasive species. The goal of the present work was to assess if the material composition of beached anthropogenic litter is indicative of the rafting fauna in a coastal area and could thus be used as a simple and cost-efficient tool for risk assessment in the future. Beached anthropogenic litter and attached biota along the 200 km coastline of Asturias, central Bay of Biscay, Spain, were analysed. The macrobiotic community attached to fouled litter items was identified using genetic barcoding combined with visual taxonomic analysis, and compared between hard plastics, foams, other plastics and non-plastic items. On the other hand, the material composition of beached litter was analysed in a standardized area on each beach. From these two datasets, the expected frequency of several rafting taxa was calculated for the coastal area and compared to the actually observed frequencies. The results showed that plastics were the most abundant type of beached litter. Litter accumulation was likely driven by coastal sources (industry, ports) and river/sewage inputs and transported by near-shore currents. Rafting vectors were almost exclusively made up of plastics and could mainly be attributed to fishing activity and leisure/ household. We identified a variety of rafting biota, including species of goose barnacles, acorn barnacles, bivalves, gastropods, polychaetes and bryozoan, and hydrozoan colonies attached to stranded litter. Several of these species were non-native and invasive, such as the giant Pacific oyster (*Crassostrea gigas*) and the Australian barnacle (*Austrominius modestus*). The composition of attached fauna varied strongly between litter items of different materials. Plastics, except for foam, had a much more diverse attached community than non-plastic materials. The predicted frequency of several taxa attached to beached litter significantly correlated with the actually observed frequencies. Therefore we suggest that the composition of stranded litter on a beach or an area could allow for predictions about the corresponding attached biotic community, including invasive species.

OPEN ACCESS

Citation: Rech S, Borrell Pichs YJ, García-Vazquez E (2018) Anthropogenic marine litter composition in coastal areas may be a predictor of potentially invasive rafting fauna. PLoS ONE 13(1): e0191859. https://doi.org/10.1371/journal.pone.0191859

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Introduction

Since plastics have been made available to a broad spectrum of consumers after the Second World War, their global production has risen to 322×10^9 kg in 2015 [1]. Although plastic production is concentrated in China, Europe, the USA, Canada and Mexico, plastics and recyclable plastic waste, which are not classified as hazardous [2], are exported internationally [1,3,4], posing a global threat to human health, interests, and ecosystems [2,5]. The pollution by plastic litter has advanced to such a level that today it is present in virtually every environment and every location of the Earth [6,7]. The marine environment is especially affected, as it receives not only direct pollution from sea-based activities, but also land-based plastics [7–9]. Plastic pollution causes the death of a high number of marine animals, as well as severe damages to ecosystems and human health and interests, like tourism, fishing, or leisure activities at beaches [10–13]. Plastics do not degrade naturally but fragment to smaller pieces, which multiplies their abundance [6]. In recent decades, campaigns are being conducted to combat the excessive production and consumption of single-use plastics, for example plastic bags from supermarkets, microbeads in cosmetic products, or PET (Polyethylene terephthalate) beverage bottles (e.g. http://storyofstuff.org/, http://www.beatthemicrobead.org/). Policy changes have been requested after increasing scientific evidence and public awareness about the pollution problem [14,15].

While research and actions on several aspects of the plastic litter problem are steadily advancing, there are still many important aspects that have gained little scientific attention so far. One problem that has received less attention is the role of anthropogenic litter items serving as artificial rafts for non-native and possibly invasive species. Notably, rafting has been mentioned in several publications [16] and public media, but at present there is no clear understanding of the scale and the underlying processes of this phenomenon. Research priorities include an estimation of its global impact, the localization of natural sink areas, and the identification of high-risk anthropogenic litter items/materials and sources [17].

Rafting of biota on floating objects, like driftwood, macro algae or volcanic pumice has importantly shaped the species composition of islands [16,18,19]. Floatable litter items of anthropogenic origin greatly enhance the number of stable rafts, particularly in areas where natural vectors are scarce. Anthropogenic litter pollution is estimated to double marine rafting opportunities [16,20] and on some beaches more than 60% of all anthropogenic litter items carried attached organisms [6]. Although the vast majority of anthropogenic litter used as rafts are plastic items, there are also cases of macrobiotic rafting on glass, metal, and paper objects [16]. Notably, a metal gas cylinder encrusted by the stony coral *Favia fragum* had probably crossed the Atlantic Ocean from the USA to the Netherlands [21]. Another invading coral, *Oculina patagonica*, is commonly found on submerged metal objects [22], while some pelagic barnacles are frequently recorded on glass and metal objects [23]. Biofouling was also reported for air-filled glass floats, used in (mainly Japanese) fisheries before plastics became widely available and still afloat in the world´s oceans nowadays [21,24–26].

Differences between materials in the abundance and composition of the micro fauna in early stages of biofouling have been found [27,28]. Particularly, polystyrene seems to carry a higher number of both species and individuals than other types of plastics, which may be due to its higher surface roughness [27,29]. Settlement of individuals of the invasive species *Bugula neritina* was significantly higher on several plastic surfaces [Polyvinylchloride (PVC), Polypropylene (PP), Polycarbonate (PC), Polyethylene terephthalate (PET) and Polystyrene (PS)] than on glass surfaces, under both field and laboratory conditions, whereas the invasive barnacle *Austrominius modestus* settled more on glass than on plastic surfaces (tested under field conditions) [30]. In contrast, no significant differences between biofilm composition on PET and glass surfaces were found in another study and object softness, rather than the type of material, was suggested to be an important factor for biota attachment [31]. On the other hand, laboratory experiments and controlled field studies with fixed floaters do not incorporate the buoyancy or floating behaviour of the different materials, which may also influence the biotic colonization by some taxonomic groups [16,27,29,32]. The ability of items to float over long distances depends not only on their buoyancy, but also on their stability and shape, with thinner and more flexible plastic items (like plastic bags and packaging material) sinking faster than thicker and more robust plastic items [33].

The origin of litter could have an influence in the attached biota. Marine anthropogenic litter stems from various sources, like households, beach-based leisure activities, sea-going activities, industries, and sewage [34]. The contribution of each source to anthropogenic litter has been investigated at many locations [9,35–37], but the main sources of litter rafts with biota are less known. For particular items, macroscopic attached biota has been reported. Examples are lines, ropes, nets and bait pots [38–40], aquaculture and other buoys [39,41], plastic packaging bands used in Antarctic bases and fishing boats [42], virgin plastic pellets [43], glass bottles [39], a gas cylinder reported above [21], a plastic spool [40], and tennis shoes and slippers [44], amongst others. Those reports might point to a higher contribution of litter items originated from sea-based activities such as aquaculture and fisheries. However, this first impression needs to be investigated in depth and on a larger geographic scale.

Floating objects displace along with currents and tides, thus their role in the dispersal of attached species may be important. Rafting on marine litter has been suggested to be involved in regional dispersal of several invertebrates [23,45,46]. For example, juveniles of the bivalve *Pinctada imbricata* and adults of *Isognomon bicolor*, which are considered invasive in Brazil, were found attached to anthropogenic litter for the first time at the Uruguayan coast, where they are regarded as potentially invasive as well [38,44]. In the Spanish part of the Bay of Biscay, several alien invasive species are registered [47], some of which are already known to attach to floating anthropogenic litter in other regions [16]. The invasive pygmy mussel *Xenostrobus securis* was first reported in the Bay of Biscay in 2012, attached to natural as well as plastic and metal objects, among others [48]. The invasive *Crassostrea gigas* and the exotic *Ostrea stentina* were also found attached to artificial materials on regional ports [49]. According to EU Regulation (EU) No 1143/2014 there are about 12,000 alien species in European countries, of which 10–15% are regarded as invasive and pose a serious threat to the environment and human interests [50]. Such species can be regarded as ecosystem infestations or epidemics, with the anthropogenic litter carrying it, being infested vectors.

Given the concern of anthropogenic beach litter our goal was to determine whether the composition of anthropogenic beach litter can predict macrobiotic communities attached to stranded litter items in a region. In answering this goal, we had three main objectives. First, determine which native, non-native, and potentially invasive macroscopic animal species are present on stranded anthropogenic litter items. Second, determine the principal material and sources of the infested vectors. Third, test if the occurrence of a certain species/ taxon can be predicted based on the general litter composition at a beach or a coastal area.

Material and methods

No specific permissions were required for sampling because all the organisms analysed in this study were obtained from litter items. Those items must be removed from the beaches as they are not natural substrate. The field studies did not involve endangered or protected species.

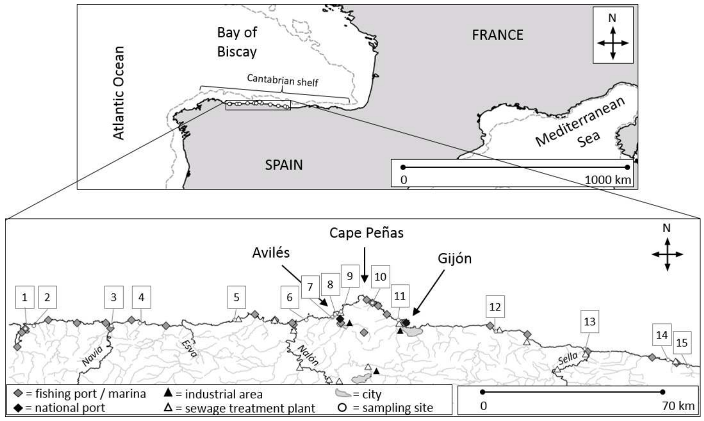

Fig 1. Map of the sampled area including waterways, national ports, fishing ports and marinas, sewage treatment plants, and principal industrial sites. Sampling sites are numbered and are specified in Table 1.

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Sampling area

To address our main research goal and objectives, we evaluated the coast of Asturias region in the south-central Bay of Biscay (north of Spain). The coast is under the influence of currents going eastwards [51], with a boundary in Cape Peñas (central cape marked in Fig 1) that divides the coast into the colder west and the warmer east zone [52]. The sampling sites cover a wide spectrum of factors that may influence marine litter distribution, like land-use, distance to human settlements, industry, and geomorphology [53–55]. There are two international cargo ports in the sampled area (Gijón and Avilés), as well as shellfish aquaculture areas in two estuaries (Ría del Eo and Villaviciosa). There are several villages and two bigger cities, Gijón and Avilés, along the coastline in Spain (Fig 1). The central area of the region is strongly polluted by industrial activities [56,57], which are mainly based in the area of Avilés. Among the several rivers discharging into the Cantabrian Sea in the sampling area, the rivers Nalón, Navia, Sella, and Esva have the largest stream basins (Fig 1).

Table 1. Sampled beaches as shown in Fig 1, with geographic position.

Number	Beach name	Longitude [˚W]	Latitude [˚N]
1	Figueras	-7.02	43.54
2	Penarronda	-6.99	43.55
3	Navia	-6.72	43.55
4	Barayo	-6.62	43.56
5	Silencio	-6.29	43.57
6	Bayas	-6.04	43.57
7	Salinas	-5.95	43.58
8	Nieva	-5.94	43.59
9	Xagó	-5.92	43.60
10	Bañugues	-5.81	43.63
11	Xivares	-5.72	43.57
12	Rodiles	-5.38	43.53
13	Sta. Marina	-5.07	43.47
14	Poo	-4.78	43.43
15	Andrín	-4.71	43.41

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Beach litter samplings and analysis

A total of fifteen sandy beaches, covering a linear distance of 190 km along the Cantabrian coastline in Asturias, Spain, were sampled in a 26-day period between February and March 2016 (Fig 1). Each beach was sampled one day during low tide and daylight. We conducted two independent surveys: 1) A sampling of fouled beached items along the whole area of each beach to test if there are material-related differences in the taxonomic composition of the macro fauna attached to beached litter, and 2) a count and material-based classification of beached anthropogenic litter in general (both fouled and non-fouled) in a smaller standardized area. Please see the supporting figure for a graphic sampling scheme (S1 Fig).

Survey 1: The whole area of each beach was searched for anthropogenic litter items with attached macrofauna (visible fauna). Each of the items found was photographed with a Motorola Moto G3 camera (resolution 13 MP) next to a size reference (a finger or any other object of known dimensions) and given an identification code. The type of object (e.g. buoy, fragment, rope; Table 2), type of material and colour was noted down for each item. We did not only classify the fouled items by material as plastic and non-plastic (e.g. metal, paper, glass; abbreviated NPl), but moreover separated plastic items in three categories, based on their stability and surface roughness: Hard plastics (abbreviated HPl), synthetic foams (e.g. Polystyrene; abbreviated foams), and other plastics (abbreviated OPl). Litter items found on the beaches were associated to three sources: Sewage, Fishing/Aquaculture and Household/Leisure. All objects or fragments that were not identifiable or not attributable to one of the categories above were classified as N/A (not attributable; Table 2).

Attached biota was visually assigned to the most specific distinguishable taxonomic group based on morphology and the number of individuals (colonies for bryozoans and hydrozoans) was counted and noted down for each group. A representative number of individuals (≤ 50) of each morphotype was detached from each litter item using forceps and a scraper. They were stored in commercially available hard plastic sampling pots in 50–500 ml (depending on the size and number of stored individuals) of ethanol 80% for further analysis and labelled with the identification code of the corresponding litter item. Some smaller litter items and items of complex shapes were stored in plastic bags and taken to the laboratory for measurement, while the dimensions of bigger items and of items with a simple shape were estimated based on the photos, and the surface area was calculated for each item. The native distribution area and the potential invasive capacity of each attached species were examined from relevant current literature [49,58–62] and databases, namely the global invasive species database (GISD, http://www.issg.org/database) and World Register of Marine Species [63].

Table 2. Categories of beach litter sources and associated litter objects.

Sewage	Fishing/Aquaculture	HH/Leisure	N/A
Cotton buds	Buoys	Sandals	Fragments
Menstrual hygiene	Netfloats	Cosmetics container	Unknown objects
products + packaging	Cage nets	Shoes	Boxes
Wet wipes	Jerrycans	Shoe soles	Bottles
	Nets	Cigarette stubs	Buckets
	Ropes	Lighters	Lids
		Paper and carton	Beverage crates
		Textiles	
		Drinking straws	

HH = household, N/A = not attributable.

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Survey 2: A standardized quantification and characterization of anthropogenic beach litter (not restricted to fouled objects) was done at all beaches, except for Figueras, Silencio, and S. Juan de Nieva (for location of the beaches see Fig 1). On the other 12 beaches, of similar sandy granulation, standardized litter counts were conducted in 2 horizontal transects at every beach, each consisting of four adjoined quadrats of 3×3m^2 each. The two transects were placed parallel to the water line, the upper transect along the most recognizable higher tideline, and the lower transect along the most recognizable lower tideline, to account for possible differences in litter composition with shore height [64] and to include both recently stranded litter (lower tide line) and litter stranded less recently (most recognizable high tide line). The area for the counts was defined at every beach after visual inspection, where accumulation of flotsam (both natural and anthropogenic) was representative of the whole beach (i.e. neither exceptionally high, nor exceptionally low, relating to the rest of the beach). This method was chosen over a random approach to avoid bias due to the small transect area (36 m^2 per transect) and the limited number of replicates (two transects per beach), as anthropogenic litter and other flotsam is often distributed heterogeneously along the beach [64,65].

The sampling quadrats were defined with a tape measure and their outlines were marked in the sand using a stick. In each quadrat all macro litter (items and fragments bigger than 1.5 cm) was inspected and sorted by object type (e.g. lid, drinking straw, fragment) and material. Then the number of items of each combination of object type and material (e.g. hard plastic lids, metal lids, paper fragments; Table 2) was counted and noted down for each quadrat in situ. All items and fragments were then assigned to a source category. The material categories and source categories used for classification were the same as described above for Survey 1.

Genetic barcoding

DNA was extracted from a small piece of tissue (about 2×2 mm) using Chelex (Bio Rad BT Chelex® 100 Resin). For DNA extraction from very small individuals with non-tissue parts, like shells (e.g., molluscs), the complete individual was treated with E.Z.N.A® Mollusc DNA Kit. PCRs were performed with the universal primers detailed in Table 3. When necessary, the PCR product was purified using EUR$_x$® Gene Matrix Agarose Out DNA Purification Kit. DNA sequencing was performed by Macrogen Europe, Amsterdam, Netherlands.

Sequence editing and alignment was done using the freeware BIOEDIT Version 7.2.5 [66]. From the DNA Barcode the species was assigned using the BLAST database [67] and the best match with the maximum hit score (minimum 97% nucleotide identity). Phylogenetic trees for confirming species assignation were built with MEGA 7 [68] from the sequences obtained in this study and reference sequences of voucher specimens taken from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/), based on the maximum likelihood reconstruction method, with 500 bootstraps.

Table 3. Primers used for DNA amplification in different taxa.

Taxon	Primers	Sequence
Molluscs, Arthropods	jgLCO1490 jgHCO2198	5′TITCIACIAAYCAYAARGAYATTGG3′ 5′TAIACYTCIGGRTGICCRAARAAYCA3′
Polychaetes	18s EukF 18s EukR	5′WAYCTGGTTGATCCTGCCAGT3′ 5′TGATCCTTCYGCAGGTTCACCTAC3′
Bryozoans Hydrozoans	16s HF 16s HR	5′ATAACACGAGAAGACCCT3′ 5′CCCRCGGTCGCCCCAAC3′

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Statistical analysis

Analysis of rafting fauna was done at regional level after confirming large dispersal capacity of the species found. Comparison among materials for the attached biotic community was done using the number of individuals per object as a standardized unit. To compare among communities we classified biota as goose barnacles, acorn barnacles, bryozoan and hydrozoan colonies, decapods, molluscs and polychaetes.

Composition and sources of beach litter found along the main accumulation lines (from standardized samplings) were compared to composition and sources of the litter items used as rafts, employing the PERMANOVA function of PRIMER 6 software [69,70]. PERMANOVA results were regarded as statistically significant at a p-value of ≤ 0.05. The contribution of each litter source to the differences was tested by SIMPER (= similiarity percentage) analysis. Both analyses were based on Bray- Curtis similarities.

The abundance of anthropogenic litter was compared between and within beaches using boxplots, showing the mean value, quartiles and variability for each beach. Heterogeneity in composition and abundance of anthropogenic beach litter in general, and of items used as artificial rafts by biota, were tested using PERMANOVA, based on Euclidean distances. Multidimensional scaling (MDS) based on Bray-Curtis similarities was used to graphically represent the grouping of the sampled beaches, based on dominant litter material: beaches dominated by hard plastics (termed HPl–dominant), beaches dominated by other plastics (termed OPl-dominant), and beaches with mixed litter composition and less than 25 litter items in the standardized sampling area ($>$ 0.35 items×m^2; termed Mix). These analyses were done for the subsample of beaches where standardized litter analysis was carried out.

Since litter composition and litter with rafting biota in a beach were independent datasets, a correlation approach was followed to determine if rafting biota in a beach area can be inferred from litter composition. Biota expectation from litter composition was estimated for 12 beaches based on the characteristic community profile of the beaches' litter materials. The goodness of adjustment between estimated and observed taxa was tested using a correlation approach, based on Spearman´s rank correlation coefficient and the linear correlation was graphically illustrated in a scatter plot.

We calculated the expected number of individuals by taxa at each of the twelve beaches as:

$$TB(x) = \sum_{i=1}^{n} fM(i) * fTBM(i, r) * Nt(x) \quad (1)$$

Where *TB* (x) is the expected number of individuals for taxon B on beach x, fM(i) is the frequency of litter material *i* (HPI, OPI, Foams or NPI) found on beach x, *fTBM* (i, r) is the frequency of taxon B on material i in the region *r* and *Nt (x)* is the total number of rafting biota found on beach *x*.

Results

Standardized quantification and categorization of anthropogenic beach litter

All the sampled beaches were polluted with anthropogenic litter. The mean abundance of anthropogenic litter ranged from 0.17 ± 0.21 items×m^{-2} (Barayo) to 5 ± 3.95 items×m^{-2} (Xivares). The abundance of anthropogenic litter varied strongly, not only between beaches, but also between quadrats within beaches, indicating a patchy distribution (Fig 2). The composition of beached litter in the region was not significantly different of the composition of litter rafts with biota (Table 4: PERMANOVA 1).

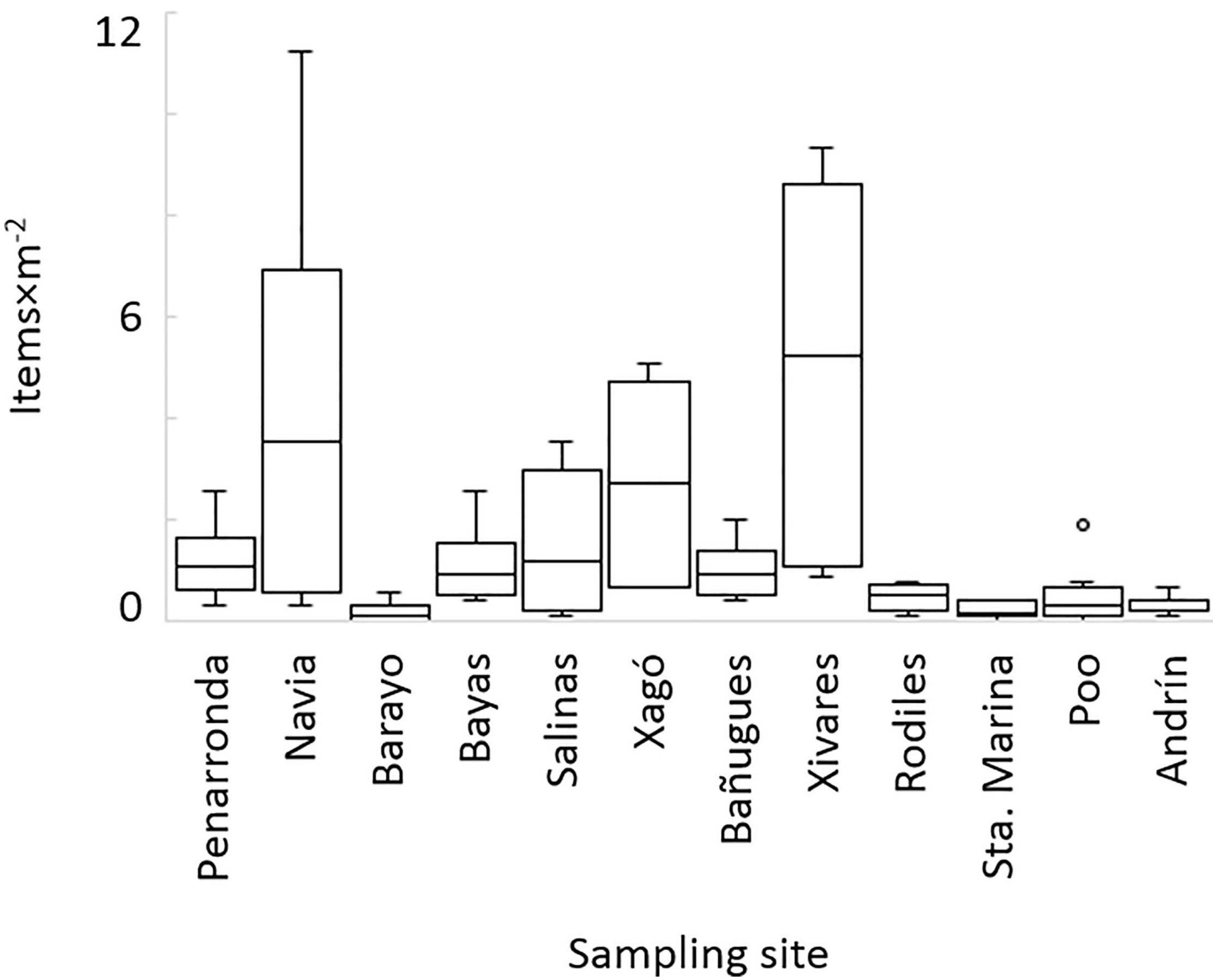

Fig 2. Abundance of anthropogenic litter, counted in a standardized area at the sampled beaches. Data are presented in a box-and whisker plot, with the middle box representing 50% of the values and the upper and lower whiskers representing the values outside of the 50% range. The median and outliers are indicated by a middle line and a circle (◦), respectively. Litter items were counted in a standardized area at each beach.

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The highest pollution levels were found in direct proximity to the coastal region´s main industrial and populational centers, Gijón (Xivares beach: 5 ± 3.95 items×m^{-2}) and Avilés (Salinas and Xagó beaches: 2 ± 1 items×m^{-2} and 2.7 ± 1.9 items×m^{-2}, respectively) both of which have a national port and a sewage treatment plant, as well as at the river mouth of the Navia river, in proximity to a fishing port and a marina (Navia beach: 4.3 ± 4 items×m^{-2}, see map in Fig 1). The abundance of beach litter at the other sampled beaches along the Cantabrian coastline seems to reflect the geomorphology of the coastline and its exposure to the prevailing eastward surface current, with a maximum peak in the northernmost Cape Peñas: Pollution rose from Barayo eastwards up to Xagó, situated on the western side of Cape Peñas, which is more exposed to the eastward surface current, and subsequently declined on the eastern side of the cape, which is more protected from the prevailing currents (Fig 1, Fig 2).

Table 4. Detailed results of PERMANOVA analyses.

PERMANOVA	Variable	Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
1	Material composition	General litter vs Rafts	1	809.71	809.71	2.6639	0.073	998
		Residuals	22	6687	303.96			
		Total	23	7496.7				
2	Litter abundance,	Beaches	11	382.66	34.788	8.7906	0.001	998
	composition	Residuals	84	332.42	3.9574			
		Total	95	715.08				
3	Litter abundance,	Beach groups	2	224.28	112.14	21.2500	0.001	999
	composition	Residuals	93	490.8	5.2774			
		Total	95	715.08				
4	Litter source	General litter vs Rafts	1	5573.5	5573.5	6.1282	0.003	995
		Residuals	22	20009	909.49			
		Total	23	25582				
5	Attached biota	Raft material	3	30743	10248	2.7185	0.001	998
		Residuals	87	3.2795E5	3769.5			
		Total	90	3.5869E5				

Df = degrees of freedom, SS = sum of squares, MS = mean sum of squares, Pseudo-F = F value by permutation, perm = permutation.

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Plastics (including foams) made up the highest share of anthropogenic litter on all beaches (75% to 100%), except at Andrín beach, where non-plastic litter was more abundant (55%; Table 5). The sampled beaches differed significantly from each other regarding both abundance and composition of anthropogenic litter (Table 4: PERMANOVA 2). Beaches were classified based on the prevalent litter material, forming three groups in the sampling area that significantly differed from each other (Table 4: PERMANOVA 3) and could be graphically distinguished by multidimensional scaling (MDS; Fig 3). The treatment of beaches in categories facilitated further analyses.

Most anthropogenic litter items found on the sampled beaches could not be attributed to a source, as many of them were small fragments. For the objects that could be likely assigned to a source, most were sewage-related. At Xagó and Penarronda fishing and aquaculture activities were also important sources of beached litter (Table 5).

Anthropogenic litter items used as rafts

A total of 94 litter objects with attached fauna were found on the surveyed beaches (Fig 4). High prevalence of hard plastics and plastics in general (71 ± 30% and 98 ± 6%, respectively), was found among rafting vectors, while the share of non-plastic objects was very low (2 ± 6%, Table 5). In fact, only five non-plastic objects with attached fauna were found on three beaches: three glass bottles (one with a metal cap), one piece of processed wood, and one sandal, which was counted as nonplastic as the attached organism was found on its textile part. Within the plastics the share of other plastics tended to be less abundant in rafting vectors than in general beach litter (17 ± 24% versus 27 ± 26%), while the share of foams was rather similar in rafting vectors and general litter (9 ± 12% and 9 ± 8%, respectively). The standard deviation between beaches however was high (Table 5).

The main sources of fouled litter items were significantly different from the main sources of other non-fouled beach litter (Table 5, Table 4: PERMANOVA 4). SIMPER showed that the source category with the highest contribution to the differences (after unidentified litter NA, contribution: 37%) was Fishing and Aquaculture (contribution: 34%; Table 6). This

Table 5. Composition and likely source of anthropogenic beach litter from standardized beach litter counts (in white, at left), and fouled litter items along the whole beach area (in grey, at right).

		Anthropogenic beach litter (from standardized sampling in quadrats)										Fouled items (from whole beach area)									
			MATERIAL [%]					SOURCE [%]				MATERIAL [%]					SOURCE [%]				
Beach group	**Beach**	**Litter [items *m^{-2}]**	**HPl**	**OPl**	**Foam**	**NPl**	**ΣPl**	**S**	**F**	**HH**	**N/A**	**Fouled vectors [total]**	**HPl**	**OPl**	**Foam**	**NPl**	**ΣPl**	**S**	**F**	**HH**	**N/A**
Mix	Andrín	**0.31**	23	5	18	**55**	45	0	0	0	100	**5**	40	40	20	0	**100**	0	20	**60**	20
Mix	Sta. Marina	**0.22**	44	6	31	19	**81**	6	0	**13**	81	**2**	50	50	0	0	**100**	0	0	**50**	50
Mix	Barayo	**0.17**	58	17	0	25	**75**	0	**8**	**8**	83	**5**	40	20	40	0	**100**	0	**40**	0	60
OPl-dom	Xagó	**2.68**	18	74	8	0	**100**	**34**	**24**	0	41	**4**	75	0	25	0	**100**	0	**75**	0	25
OPl-dom	Penarronda	**1.25**	43	54	2	0	**100**	3	**42**	0	54	**20**	75	10	10	5	**95**	0	**25**	10	65
OPl-dom	Bayas	**1.15**	41	45	10	5	**95**	**8**	4	2	86	**7**	71	14	14	0	**100**	0	**33**	0	67
OPl-dom	Navia	**4.25**	26	68	1	6	**94**	**17**	5	6	72	**1**	100	0	0	0	**100**	0	**100**	0	0
HPl-dom	Salinas	**1.50**	80	11	7	2	**98**	**13**	3	3	81	**25**	48	20	20	12	**88**	0	4	**12**	84
HPl-dom	Xivares	**5.00**	89	3	8	1	**99**	**18**	2	0	80	**4**	75	25	0	0	**100**	0	**25**	**25**	50
HPl-dom	Bañugues	**1.03**	72	14	12	3	**97**	**9**	7	5	78	**2**	100	0	0	0	**100**	0	0	0	100
HPl-dom	Rodiles	**0.47**	71	21	6	3	**97**	**9**	6	3	82	**8**	88	0	13	0	**100**	0	**75**	0	25
HPl-dom	Poo	**0.50**	78	11	8	3	**97**	**19**	3	6	72	**1**	100	0	0	0	**100**	0	0	0	100
X	Silencio	**x**	x	x	x	x	x	x	x	x	x	**4**	100	0	0	0	**100**	0	**25**	0	75
X	Nieva	**x**	x	x	x	x	x	x	x	x	x	**1**	100	0	0	0	**100**	0	0	0	100
X	Figueras	**x**	x	x	x	x	x	x	x	x	x	**5**	0	80	0	20	**80**	0	0	**20**	80
	MEAN		54	27	9	10	**90**	**11**	9	4	76		71	17	9	2	**98**	0	**28**	12	60
	ST. DEV.		24	26	8	16	16	10	12	4	15		30	24	12	6	6	0	32	19	32

x = no data available. dom = dominant, HPl = Hard plastics, OPl = Other plastics, NPl = Nonplastic, S = Sewage, F = Fishing and aquaculture, HH = Household and leisure, N/A = Not attributable, ST. DEV = Standard deviation.

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particularly important role of fishing/aquaculture related litter for the rafting of biota in the sampling area was especially noticeable at the beaches of Xagó, Navia and Rodiles, where all the identifiable items with attached biota were from this source (Table 5). Leisure and household-related items also had a high share in rafting vectors. Items from this source were found on six beaches and consisted of 20 shoes/sandals and one cosmetic container. Leisure and household was the main litter source for Andrín beach (Table 5). On the other hand, sewage-related litter made up to 11% (mean) of all anthropogenic beach litter, although none of the biota rafts was related to this source (Tables 5 and 6).

Fauna attached to anthropogenic rafts

More than 3300 individuals (or colonies for bryozoans and hydrozoans) were found attached to the litter objects found in the beaches surveyed (Table 7). With genetic analyses, more than 400 DNA barcodes were obtained, identifying 23 species of attached animals from four phyla (Fig 5, Table 7). The Barcodes were submitted to GenBank database, where they are available with the Accession Numbers KY607884-KY607909, KY614195-KY614223, KY628986, KY661434-KY661534, KY683467-KY683511, KY944812-KY944984, KY963587-KY963595, KY986731-KY986745, MF037237-MF037246, MF043915. Crustaceans (Phylum Arthropoda) such as Lepadidae (Goose barnacles), Balanidae and Verrucidae (Barnacles), and the amphipod *Caprella andreae* were the most abundant animals in this study (> 1000 individuals; Table 7), followed by annelids, which all belonged to the family Serpulidae (~700 individuals).

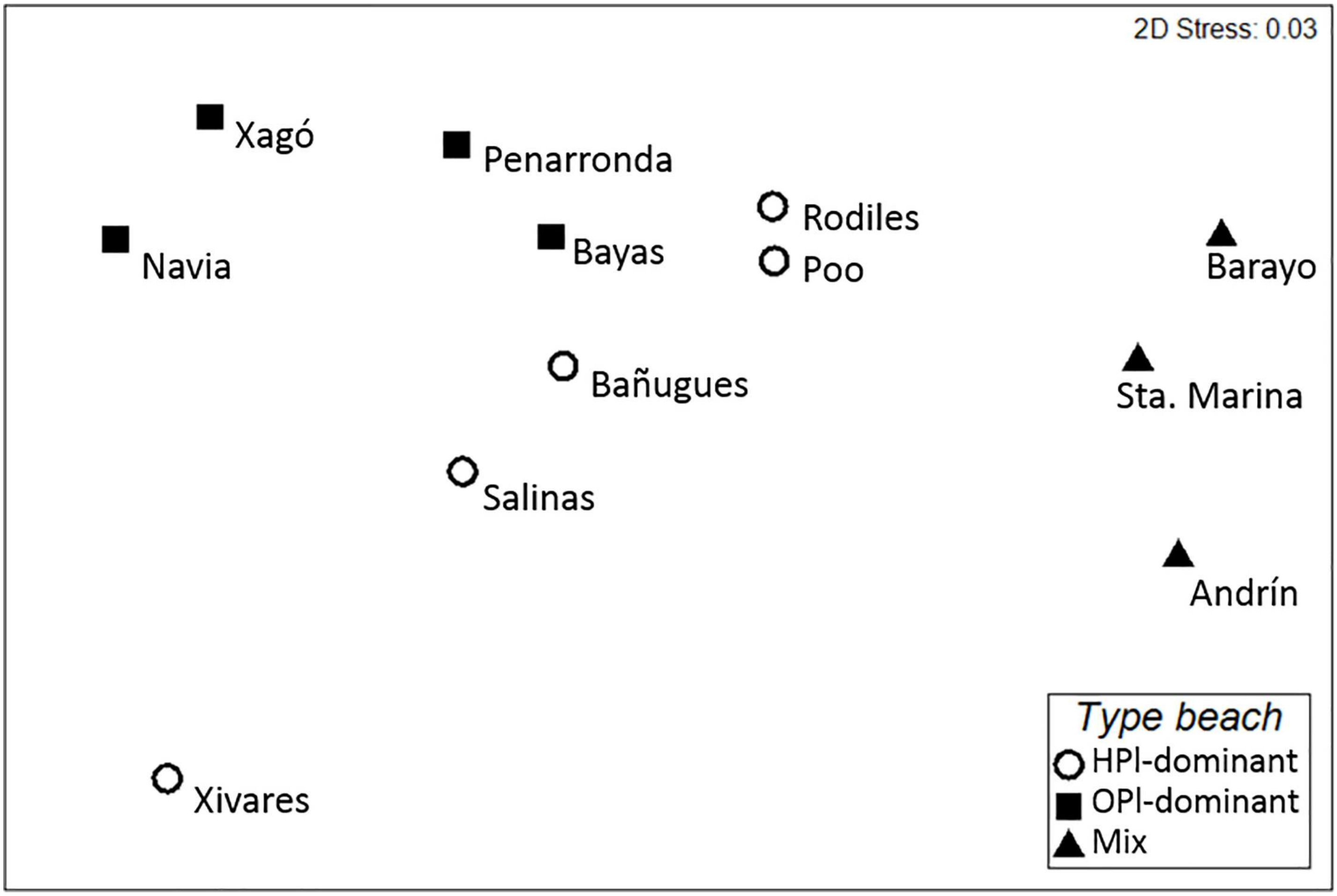

Fig 3. Multi-dimensional plot of the sampled beaches, based on abundance and composition of anthropogenic litter counted in a standardized area at each beach. HPl = hard plastics, OPl = Other plastics, Mix = beaches with mixed litter composition and less than 25 litter items in the standardized sampling area (> 0.35 items×m^2).

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Hydrozoan and bryozoan colonies were also very numerous (~400) and might be underestimated in this study, due to the difficulty of counting them individually. As most of the colonies were dried out and in a state of advanced degradation, DNA was degraded in most cases and

Fig 4. Examples of fouled litter items. a) Hard plastic object with oyster, polychaetes and acorn barnacles b) PET bottle with goose barnacles c) float of fishing net with bryozoan colonies and polychaetes, d) shoe sole with oyster, snail and acorn barnacles, e) duct tape with goose barnacles.

https://doi.org/10.1371/journal.pone.0191859.g004

Table 6. Contributions of several litter sources to the differences between the general beach litter counted in a standardized area (group General) and litter used as biota raft (group Rafts), calculated by SIMPER analysis.

						Groups General & Rafts
						Average dissimilarity = 44.61
	General	Rafts				
Litter source	Abundance	Abundance	Dissimilarity	Diss. / SD	Contribution [%]	Cumulative [%]
Not identified	75.83	53.83	16.67	1.45	37.37	37.37
Fishing/Aquaculture	8.67	33.08	15.36	1.06	34.44	71.81
HH / Leisure	3.83	13.08	6.90	0.77	15.47	87.28
Sewage	11.33	0.00	5.68	1.22	12.72	100.00

HH = household, Diss. = Dissimilarity, SD = standard deviation

https://doi.org/10.1371/journal.pone.0191859.t006

Table 7. Overview of species attached to stranded litter, identified in the present study.

Visual identification	*N*	*Phylum/ Subphylum*	*Class*	*Order*	*Family*	*Genetic identification*	*Barcodes*	*Geographic origin*
Goose barnacles	676	Arthropoda/ Crustacea	Maxillopoda	Pedunculata	Lepadidae	*Lepas anatifera*	170	COS
						Lepas anserifera	2	
						Lepas pectinata	44	
						Dosima fascicularis	3	
Acorn barnacles	308	Arthropoda/ Crustacea	Maxillopoda	Sessilia	Balanidae	***Austrominius modestus****	**57**	**Australia, NZ**
						Chthamalus stellatus	30	NAT
						Chthamalus montagui	26	
						Balanidae sp., (Perforatus perforatus)	2	
					Verrucidae	*Verruca stroemia*	4	
Caprellids	75	Arthropoda/ Crustacea	Malacostraca	Amphipoda	Caprellidae	*Caprella andreae*	10	COS
Σ ARTHROPODS	**1059**							
Mytilidae	70	Mollusca	Bivalvia	Mytiloida	Mytilidae	*Mytilus edulis*	5	NAT
						*Mytilus galloprovincialis**	1	
						Mytilus sp.	10	x
Ostreidae	21	Mollusca	Bivalvia	Ostreoida	Ostreidae	***Crassostrea gigas****	**16**	**NE-Pacific**
						Ostrea stentina	**1**	**S-Atlantic, Med**
Gastropods	2	Mollusca	Gastropoda	x	Trochidae	*Gibbula umbilicalis*	2	NAT
Σ MARINE MOLLUSCS	**93**							
Polychaetes	699	Annelida	Polychaeta	Canalipalpata	Serpulidae	*Spirobranchus triqueter*	3	NAT
						Spirobranchus taeniatus	17	
						Serpula columbiana	**1**	**N-Pacific**
						Neodexiospira sp.	**1**	**S-Atlantic**
						Spirobranchus sp.	3	x
Σ ANNELIDS	**699**							
Hydrozoan and Bryozoan colonies	396	Cnidaria	Hydrozoa	Anthoathecata	Bougainvilliidae	*Bougainvillia muscus*	1	NAT
				Leptomedusae	Campanulariidae	*Obelia dichotoma*	1	COS
Σ HYDROZOANS + BRYOZOANS	**396**							
Gastropod, terrestrial	4	Mollusca	Gastropoda	x	Helicidae	*Helix aspersa aspersa**	4	NAT

N = total number of individuals found, NAT = native, COS = cosmopolitan distribution, N = North, S = South, Med = Mediterranean sea. Non-native species are marked by bold writing.

* = Species (both native and non-native to study area) listed in the global invasive species database (GISD, http://www.issg.org/database).

https://doi.org/10.1371/journal.pone.0191859.t007

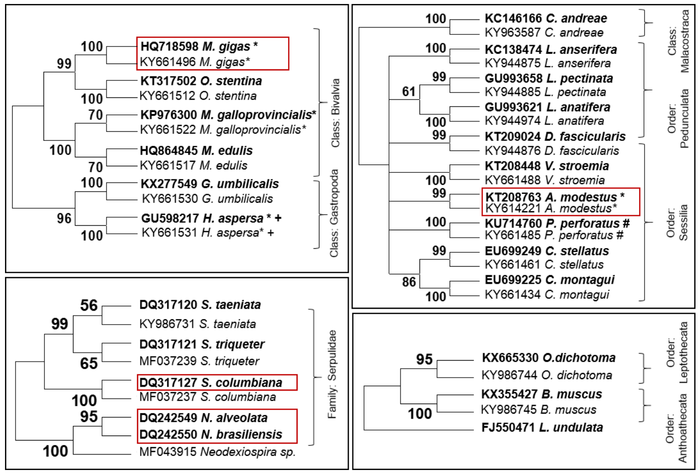

Fig 5. Phylogenetic trees reconstructed from sequences obtained in this study and reference sequences from GenBank database (bold style). a) molluscs, b) crustaceans, c) polychaetes, d) hydrozoans. Frame = Species not native to the study area; * = Species listed in the invasive species database; + = Terrestrial species; # = reference without species voucher.

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only two species of Cnidarians were identified from genetic techniques: *Bougainvillia muscus* and *Obelia dichotoma*. The animals found in the present study were morphologically diverse and it is possible that the hydrozoan and bryozoan colony group actually included more species and taxa. Around 100 molluscs were found attached to anthropogenic litter items, with the majority of them belonging to the genus *Mytilus*, followed by the oysters *Crassostrea gigas* and *Ostrea stentina*. Moreover, we found two species of gastropods: the marine species *Gibbula umbilicalis*, and the land snail *Helix aspersa*. For the latter, which is terrestrial, taking into account its common occurrence in the sampled area, it seems likely it did not arrive on the beach by rafting but from the land.

Most of the rafting animals were native to the study region or recognized as cosmopolitans (Lepadidae). Five species were not native: *Crassostrea gigas*, *Ostrea stentina*, *Austrominius modestus*, *Serpula columbiana*, and *Neodexiospira sp*. *C*. *gigas* and *A*. *modes*tus are listed in the global invasive species database (GISD, http://www.issg.org/database). The native *M*. *galloprovincialis* and the terrestrial species *H*. *aspersa* are included in GISD as well. The species identification provided by BLAST was confirmed from phylogenetic analysis after clustering analyses including voucher species references from GenBank (Fig 5).

Regarding the type of material carrying each species, differences occurred in this region between taxonomic groups. While molluscs like *Mytilus* and *Crassostrea* were found on all types of anthropogenic litter, Polychaetes were exclusively found on hard plastic and other plastic items. Barnacles, like *Austrominius*, were found on all materials except foams, but were most important on hard plastic items. Therefore, each type of litter seemed to exhibit a particular profile of attached biota (Fig 6). Foams carried almost exclusively goose barnacles (99%) and, to a much lesser extent, molluscs (1%). Non-plastic items contained a similar biota profile, with an additional small share of barnacles (2%). Hard plastic and other plastic objects on the other hand carried a broad spectrum of attached taxa. On hard plastic items the main share of attached biota were barnacles (37%), polychaetes (31%) and bryozoan colonies (18%). They also carried goose barnacles, molluscs, and decapods (7%, 4%, and 2%, respectively). On other plastics, the main share of attached biota was made up of polychaetes (66%) and goose barnacles (23%), while barnacles, bryozoan colonies, and molluscs were less common (5%, 5%,

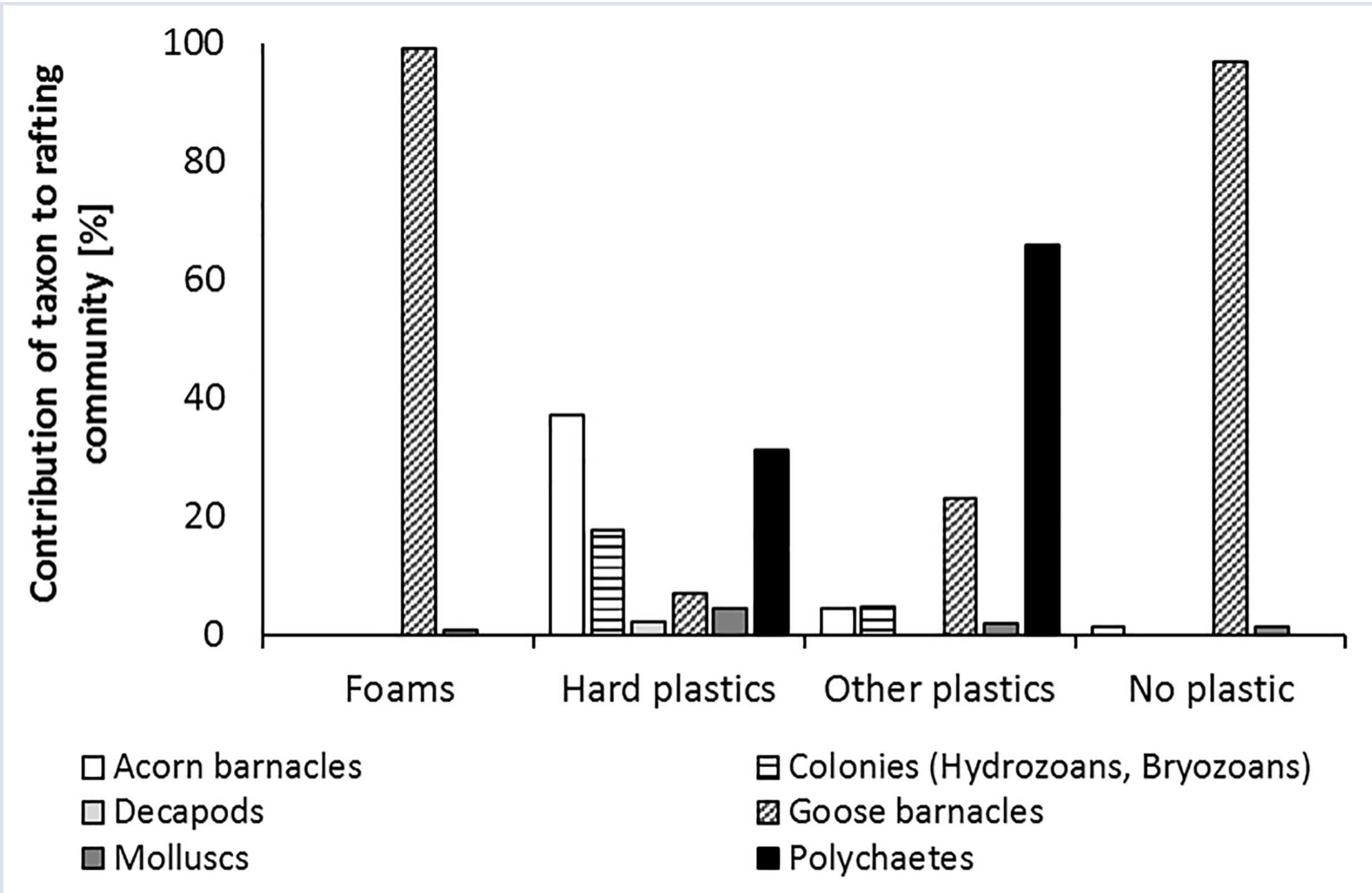

Fig 6. Particular profile of attached biota for each litter material.

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and 2%, respectively). Differences between materials regarding the biota profile were indeed highly statistically significant (Table 4: PERMANOVA 5).

Inference of litter-related biotic community from beach litter composition

We tested if the composition of an area´s macrobiotic communities attached to stranded litter items can be predicted based on its composition of anthropogenic beach litter, using the data of the 12 beaches where standardized litter counts have been conducted. The predicted frequency of attached biota of several taxa, estimated from litter composition significantly correlated with the actually observed frequencies on both sides of cape Peñas (Western side: Spearman's rank correlation coefficient (R) = 0.498; $p = 0.002$; Eastern side: R = 0.629; $p = 0.027$), as well as for the whole sampling area (R = 0.565; $p < 0.001$; Fig 7). For the exact figures of estimated and observed biota, please see the Supporting table (S1 Table).

Discussion

In this study six rafting species were recorded for the first time on anthropogenic beach litter: *Verruca stroemia*, *Ostrea stentina*, *Gibbula umbilicalis*, *Spirobranchus taeniata*, *Serpula columbiana*, and *Neodexiospira sp*. Although many rafting species have been documented on anthropogenic marine litter during the last years [16] and the recent discovery of 289 living

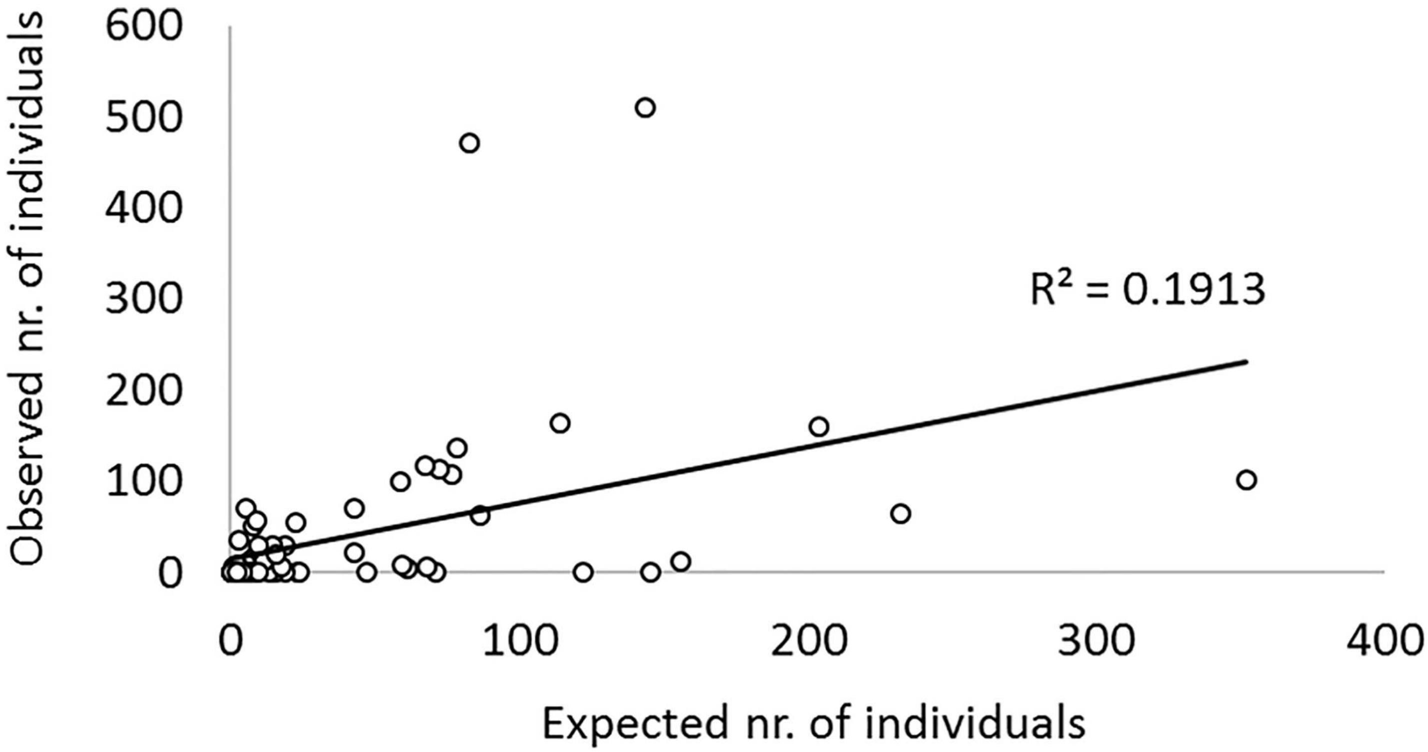

Fig 7. Correlation between expected and observed numbers of individuals of several taxa, calculated from data of 12 Asturian beaches.

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marine species, which had crossed the Pacific Ocean on objects detached by a tsunami, showed the importance of floating marine litter as a rafting vector [71], many rafting species are not known or reported yet and knowledge of the actual dimension and impact of marine litter rafting is still far from complete. The finding of *Perforatus perforatus* on anthropogenic litter is particularly interesting, as large numbers of this species, probably originating from NW Spain, have been found on beach litter in Wales [72]. A similar range expansion might also occur for invasive barnacles, such as *Austrominius modestus*.

Besides the species listed above, most of the taxa found in our study are known rafters and have already been found on anthropogenic litter (floating or stranded) in other regions [16]. The predominance of cosmopolitan stalked barnacles among marine rafters is a common phenomenon, with the small and light-weight species *L. pectinata* and *D. fascicularis* being especially suited for the colonization of smaller rafts [16,23]. *Lepas* barnacles may influence the rafting community on plastic debris: the ratio *Lepas* cover /surface area was found positively correlated with the diversity of mobile rafters, while negatively with sessile rafters' diversity, in a study by Gil and Pfaller (2016). Our results were concordant with this study, since the debris dominated by goose barnacles contained a very low diversity of other sessile rafting species (only molluscs and acorn barnacles), while materials with a lower share of goose barnacles exhibited a relatively diverse attached community (Fig 6). Another common rafter found in this study was the amphipod *Caprella andreae*. The genus *Caprella* is generally adapted to rafting because of their reduced abdominal appendages, and *C. andreae* is the only known obligate rafter in its genus [60].

Two non-native oysters were found on Figueras beach, close to the region´s only active site of mollusc aquaculture. While *C. gigas* is a recognized invasive species and quite common along the Asturian coast, *O. stentina* has only been reported in the region once before, in the port of Avilés [49]. These two findings with a linear distance of less than 100 km may indicate that this species is already established in the region, and may use anthropogenic litter for dispersal beyond the range of its propagules. The results show a link between the composition of anthropogenic beach litter in an area and the frequency of several taxa of fauna attached to stranded litter objects. This finding should be valid for a broad range of coastal regions, as it is based on taxa composition and general litter materials, rather than on particular species and/or litter items, which may vary more strongly between regions.

The strong prevalence of (hard) plastic rafts confirms the results of previous studies [73]. The very low share of non-plastic rafts may be due to the fact that the majority of these items are not buoyant and/or of very little persistence. Plastic foams, despite being highly buoyant and having rather rough surfaces, which facilitate initial colonization [16], are less stable and persistent than hard plastics [29]. This may explain their low share amongst rafting vectors. For the potential sources of litter with rafting biota, there was a high share of unidentified items but still some important conclusions may be drawn from our results. Firstly, rafting vectors could be identified and attributed to a source much more frequently than other items of anthropogenic beach litter. The reason is probably that small plastic fragments whose source cannot be identified, which are quite common in beach litter in general, are too small for serving as rafts. Fazey and Ryan (2016) proposed size and buoyancy as predictors of dispersal distance for floating debris [74]. Given that biofouling reduces an item´s buoyancy, smaller items will sink faster than bigger items and travel much smaller distances [75]. This phenomenon may also explain why sewage litter, although quite abundant on beaches, was never found as a rafting vector. Rafting vectors from fishing and aquaculture, as well as other sea-based activities, have been reported in other studies [41,76]. An explanation for the high occurrence of items from these sources among rafts may be their buoyancy, stability, size and persistence. 12 of the 23 fishing/aquaculture-related rafting vectors were buoys or netfloats, which are obviously highly buoyant and seven were grids or cages made from stable plastic wire, which are big items with a rather small surface/volume ratio. The other four rafts were rather big items (min. 10x2x2 cm^3) made from hard plastics. Leisure and household-related litter is quite difficult to define, because many of the items which might stem from this source might as well stem from sea-based sources (e.g. PET bottles). These items have not been assigned to a source category, so perhaps the actual contribution of this source was higher. Shoes and sandals, clearly sourced household or leisure, are known to be able to float over large distances and have already been reported as rafting vectors [44,77–79].

The patchy abundance of beach litter, with high variances both within and between beaches was congruent with the situation reported in many other studies [7,9,80]. Although comparisons of abundance between different locations, observers, and studies with different approaches (regarding for example transect size, choice of strand lines and/or ground between strand lines sampled, minimum size of items counted, biological material present in the sampled area etc.) are rather difficult [7,65,81], the abundance of beach litter found in this study falls within the same range as reported for many other sampling sites around the globe. As this study focuses on stranded litter which had already been at sea, the litter counts were conducted in transects targeting tidelines, where natural and anthropogenic litter is deposited by the sea. Targeting areas of litter accumulations, the results are likely overestimating the total litter abundances of the sampled beaches, and are not representative for the whole area of the beaches. They do however allow for comparisons of stranded litter abundances between the beaches sampled during this study, where the same method was used for all beaches.

Plastics (including plastic foams) are reported as the main constituents of beach litter in most studies [7]. According with that, the share of plastics found on beaches along the Cantabrian coast (present study) was rather high. Source attribution of the stranded litter items was a difficult task because the majority of items could not be clearly related to a litter category, either because the item could stem from several sources, or because the item was not identifiable (i.e. fragments). Notwithstanding it, our results indicate that sewage-related litter is a problem in the sampled area. In fact, waste-water discharging pipelines and accumulations of preproduction pellets in the sand below such pipelines were noted on several of the sampled beaches (personal observation SR), but did not enter in the present study due to their small size. Fishing and aquaculture have also been identified as important litter sources in the sampling area. This finding is consistent with the fact that pollution by lost or discarded fishing gear is a common problem in the world's seas (including the benthos) and on beaches [37,82–84]. There is a high activity of small-scale fishery, with 19 fishing ports along Asturias coastline and a large area of fishing grounds near- and off-shore, plus one active site of mollusc aquaculture (mainly oysters) near Figueras, and several crustacean ponds (http://www.sigmarinoasturias.es/).

The exposure to the prevalent currents may make the sampling area a sink for anthropogenic floating litter and attached biota from other areas. In fall and winter, the sampling area is dominated by a warm poleward surface current, referred to as 'Navidad', which enters near Cape Finisterre and moves eastward along the Cantabrian shelf and slope [51]. As the samplings presented in this study were conducted from mid-February to mid-March, it could be assumed that the overall accumulation pattern, particularly the increase of litter abundances from more western beaches towards the tip of Cape Peñas, was driven by this current. On the eastern side of cape Peñas, sediments are transported from the coastal currents to the beaches [85]. This transport may explain the observed abundances of litter on these beaches, which are not directly exposed to the prevalent current. Apart from this main driver, there seems to be an effect of rivers in the area, contributing to the high litter abundance on the beaches Navia and Xivares. Both are situated at the mouth of rivers (Rio Navia and Rio Aboño, respectively). Riverine influence was also reflected in the relatively high share of sewage-linked litter on both beaches.

Although the present study clearly showed the relation between anthropogenic beach litter composition and attached fouling biota in a coastal area, it had some limitations. The samplings were restricted to one geographic area (the south-central Bay of Biscay) and season (february to march), and each beach was sampled only once. Moreover, our study concentrated on stranded anthropogenic litter and did not include litter which was still floating in the water. Thereby we ensured to sample only taxa/species which are still present after a beaching event and might therefore pose a risk of invasion. On the other hand, it should be considered that the biota found on beach litter in this study probably do not represent the complete macrobiotic rafting community of the respective items before the beaching event, as beached litter is often biased towards sessile biota [16].

In summary, the results presented here give several important insights in the mechanisms on biota rafting on anthropogenic marine litter. Plastic items, except for foams, house a much more diverse biota community than non-plastic items and foams, which may be due to their stability and buoyancy. Several non-native and invasive species were present on litter items along the sampled beaches. Aquaculture and fishing activities were a major source of biota rafts, while sewage discharge was the most important source of all anthropogenic beach litter in the study region. We found that the frequency of a specific taxon of rafting biota in a coastal area may be predicted based on each litter material's characteristic biota profile and the beaches' litter composition. This approach, after refined and tested from more regions, could serve as a simple and cost-efficient tool for risk assessment in the future.

Supporting information

S1 Fig. Sampling scheme.
(TIF)

S1 Table. Expected vs. observed numbers of each taxon of the community attached to stranded litter.
(DOCX)

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Investigation: Sabine Rech.

Methodology: Sabine Rech.

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Resources: Yaisel J. Borrell Pichs, Eva García-Vazquez.

Supervision: Yaisel J. Borrell Pichs, Eva García-Vazquez.

Visualization: Sabine Rech.

Writing – original draft: Sabine Rech.

Writing – review & editing: Sabine Rech, Yaisel J. Borrell Pichs, Eva García-Vazquez.

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CHAPTER 3

Dispersal of alien invasive species on anthropogenic litter from European mariculture areas

Rech S, Salmina S, Borrell YJ, Garcia-Vazquez E.

Marine Pollution Bulletin 2018, 131: 10-16

Photo by Sabine Rech

Este capítulo (p. 46bis) se corresponde con el artículo:

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CHAPTER 4

Travelling light: Fouling biota on macroplastics arriving on beaches of remote Rapa Nui (Easter Island) in the South Pacific Subtropical Gyre

Rech S, Thiel M, Borrell YJ, Garcia-Vazquez E.

Marine Pollution Bulletin 2018, 137: 119-128

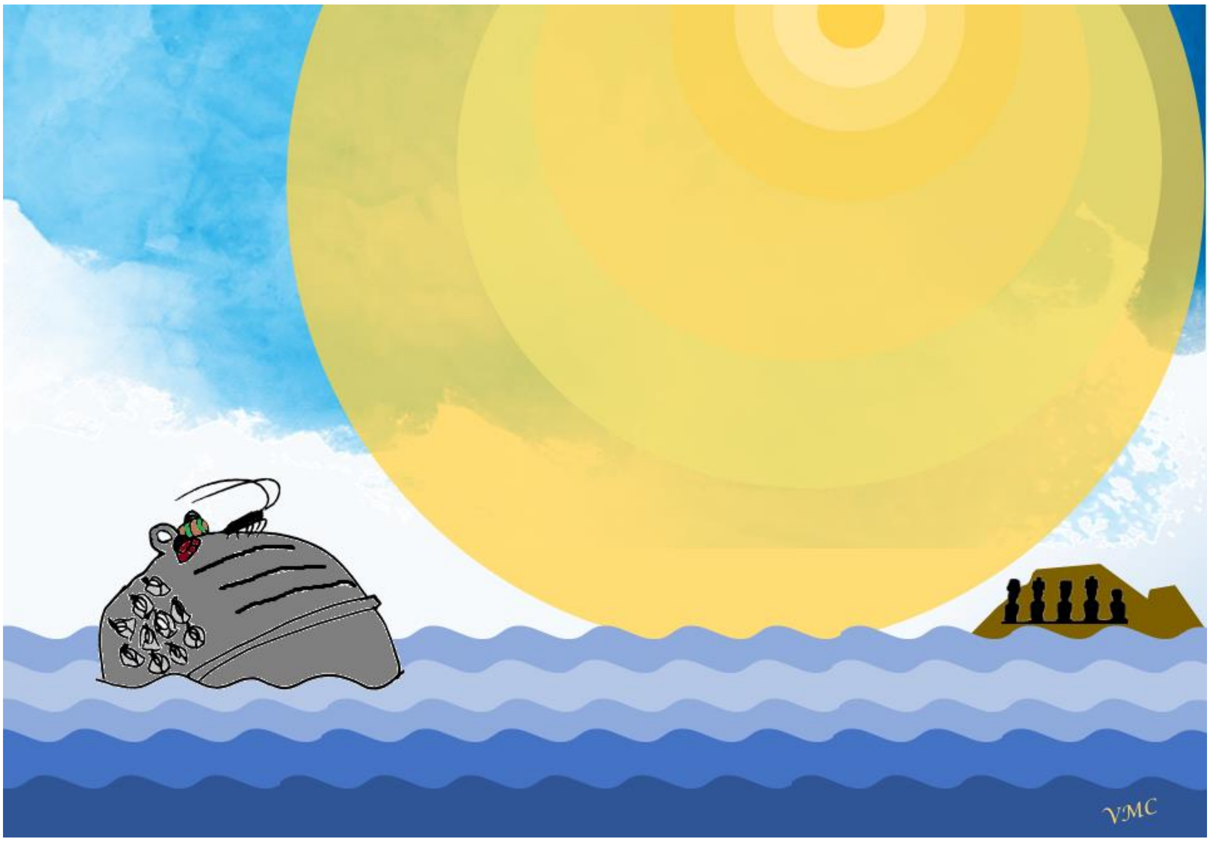

Image by Vivian Macaya, Ecoterra, Coquimbo, Chile. vmacaya.c@gmail.com

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Discussion

DISCUSSION

1. Rafting fauna

1.1. Overview of rafting fauna

Rafting biota attached to anthropogenic marine litter were found in almost all sites sampled in the course of this Thesis. Although the previous inventories of several authors (e.g. Goldstein et al., 2014; Kiessling et al., 2015; Carlton et al., 2017) list large numbers of rafting species and taxa, twelve of the biota found during the samplings had never been reported as rafting on anthropogenic material before: The serpulid polychaetes *Hydroides sanctaecrucis*, *Neodexiospira* sp., *Sabellaria alveolata*, *Serpula columbiana* and *Spirobranchus taeniata*, the molluscs *Gibbula umbilicalis, Magallata angulata*, *Mytilus edulis* and *Ostrea stentina*, as well as the barnacles *Chthamalus montagui* and *Verruca stroemia*. This is especially interesting as several of these species are non-native and invasive in the sampling areas and points out the need for more studies to understand the role of marine litter as a vector of invasive species.

The findings of this Thesis indicate that there are still many rafting species, amongst them non-native, invasive species (NIS), that remain undetected and that the actual amount of biota using floating anthropogenic litter for their transport and dispersal may be much larger than previously thought. More than 3300 individual biota as well as high densities of hydrozoan and bryozoan colonies from four geographic regions were analysed during this study. The biota found belonged to 51 taxonomic units (TU) from 6 phyla. 36 TU were successfully identified on species level (Table S1). Arthropods were the most diverse phylum, comprising 17 TU, followed by molluscs (15 TU), annelids (12 TU), cnidarians (4 TU), bryozoans (3 TU) and echinoderms (1 TU; Figure 11).

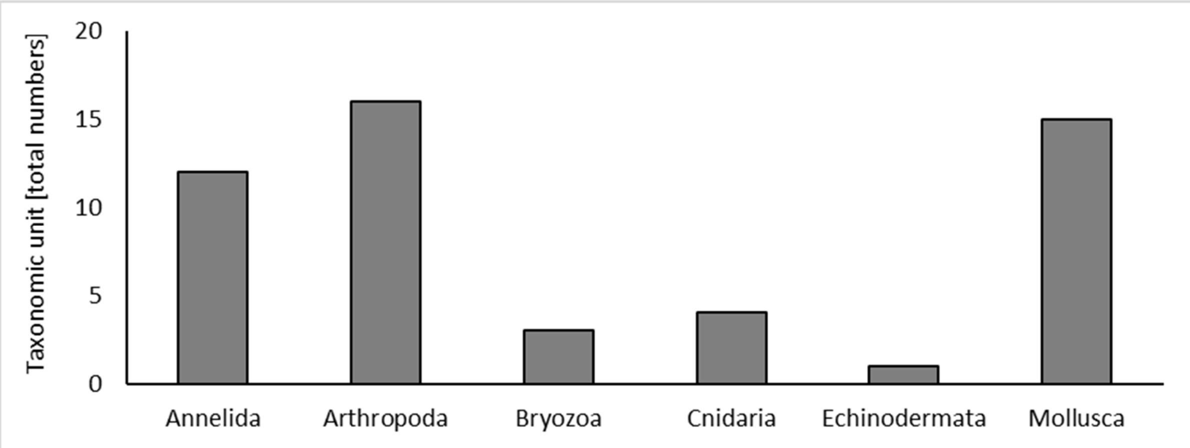

Figure 11. Taxonomic units of biota attached to stranded rafts per phylum.

Many of the species found were well-known rafters, such as *Lepas* sp., *Planes major*, or *Caprella andreae*. Some species were found attached to floating anthropogenic litter in several sampling areas: *Lepas anatifera*, for example was present in both North Atlantic sampling region (Algarve and Cantabrian), as well as in the South Pacific sampling area. The mussels *Mytilus edulis* and *M. galloprovincialis*, as well the polychaete worm *Spirobranchus triqueter* were found both in the North Atlantic and the Mediterranean. This shows that many species, even if they are not obligate rafters, are commonly transported and dispersed by anthropogenic marine litter items. Apart from the so-termed „plastisphere“, referring to microbial communities inhabiting plastic litter (Zettler et al., 2013), there seems to be a community of macrobiota, regularly inhabiting and being transported on anthropogenic marine litter. Kiessling et al. (2015) have previously stated that *„Some taxa have repeatedly been observed associated with floating litter … and thus, may not just be accidental rafters “*. This view is corroborated by the findings of this Thesis.

1.2. Regional differences in rafting fauna and rafts

The highest biotic diversity on stranded litter was found in the Cantabrian region of the North Atlantic, where 25 TU could be distinguished (Figure 12 above). 20 TU were found in each of the two aquaculture regions studied (Mediterranean and North Atlantic – Algarve).

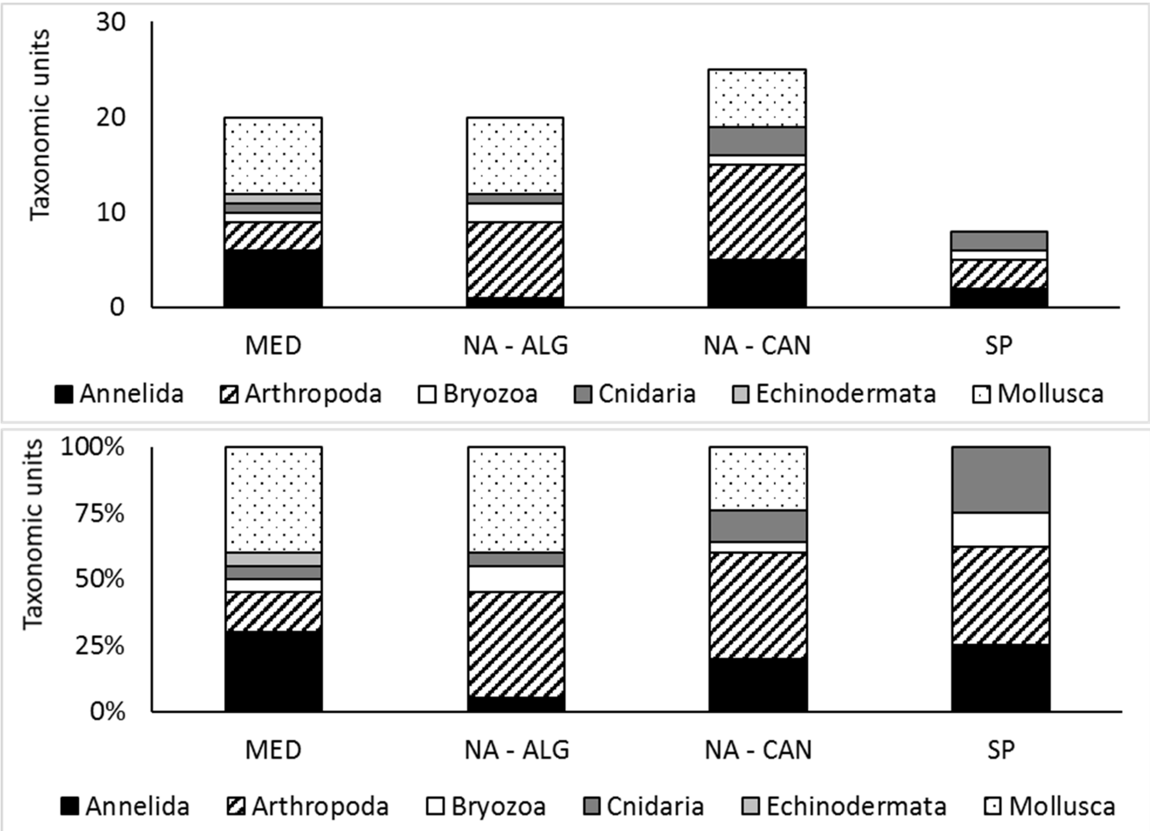

Figure 12. Above: Taxonomic units of biota attached to stranded rafts by phylum and geographical region. Below: Contribution of each phylum to the taxonomic diversity of fauna attached to stranded litter in each geographic region. MED = Mediterranean, NA = North Atlantic, ALG = Algarve, CAN = Cantabrian Sea, SP = South Pacific.

Taxonomic diversity was lowest in biota from stranded plastics of South Pacific Rapa Nui island, where only 8 TU were found. The taxonomic composition of the fauna attached to stranded items differed between geographic regions (Figure 12 below). The rafting fauna on North Atlantic and South Pacific beaches was generally dominated by arthropod species

(37.5% – 40% of taxonomic diversity), in contrast to the rafting fauna from the Mediterranean, where they only accounted for 15% of TU. Molluscs accounted for 40% of the taxonomic diversity in both aquaculture regions (NA – Algarve and Mediterranean), as well as for 24% in the North Atlantic Cantabrian region, but were completely absent from South Pacific samples (Figure 12 below). These regional differences in the rafting community of anthropogenic marine litter suggests that future studies should have a regional focus.

The regional diversity found in this study might be due, at least in part, to regional differences regarding the types of rafts. A total of 173 items (NA-CAN: 94 items, SP: 57 items, NA-ALG: 13 items, MED: 9 items) of 17 item categories (Table S2) were found with attached biota. Of these, only four categories had a contribution of ≥ 5%: PL15 (mussel bags), PL17 (fishing gear), PL19 (ropes) and PL24 (unidentified object and fragments; Figure 13).

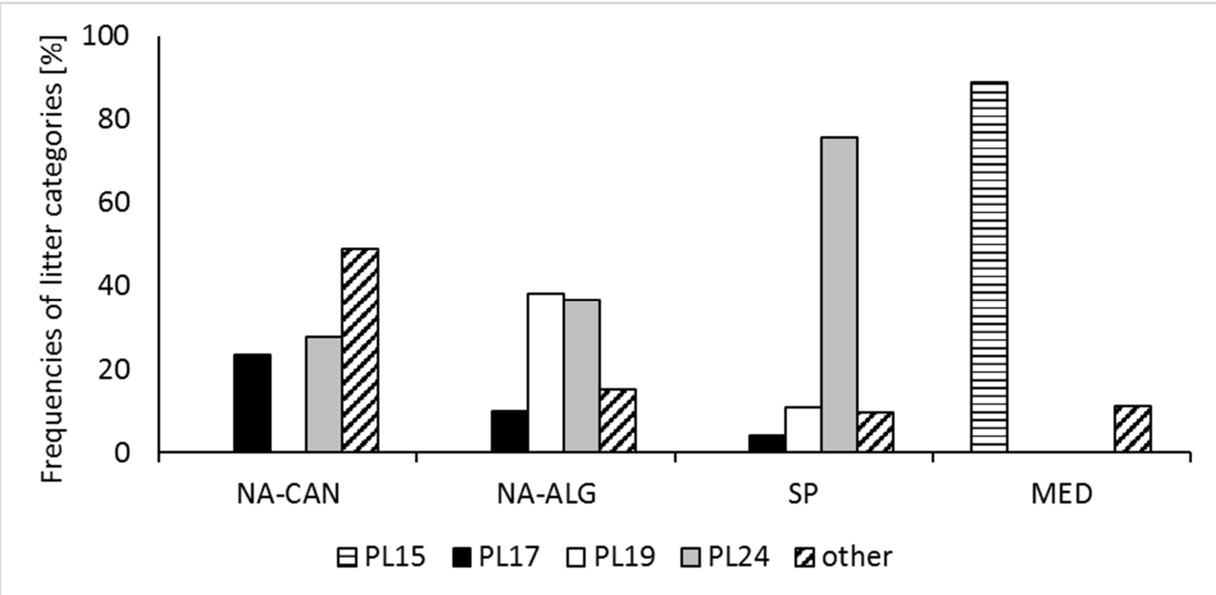

Figure 13. Frequencies of litter categories in items used as rafts, by geographic region. PL15 = mussel bags, PL17 = fishing gear, PL19 = ropes, PL24 = unidentified fragments and objects.

The composition of rafting substrata differed slightly, but statistically significantly between the three seas (North Atlantic (NA), South Pacific (SP), Mediterranean (MED); Table 1, P1). The Mediterranean differed strongly from all other sampling regions, as rafts found there were exclusively made up of two categories (PL14: plastic buoys; PL15: mussel bags), of which PL15 was not found in any other sampling site (Figure 14).

Table 1. Permutational MANOVAs (PERMANOVAs). ID = Identification, Df = degrees of freedom, SS = sum of squares, MS = mean sum of squares, Pseudo-F = F value by permutation, perms = permutations. * = significant *p*-value. Bold = Factor. Seas / Regions: North Atlantic (NA) / Cantabrian (CAN), Algarve (ALG); South Pacific (SP); Mediterranean (MED).

ID PERMANOVA	Source of variation	df	SS	MS	Pseudo-F/t	*p* - value	Unique perms.
P1 main	**Sea**	2	18411	9205.7	2.6456	0.005*	970
	Residuals	18	62633	3479.6			
	Total	20	81044				
P1 pairwise	NA, SP				1.6239	0.025*	628
	NA, MED				1.5321	0.161	17
	SP, MED				3.7388	0.241	4
P2 main	**Region**	3	22623	7540.9	2.1943	0.017*	998
	Residuals	17	58421	3436.5			
	Total	20	81044				
P2 pairwise	NA-CAN, SP				1.6916	0.012*	510
	NA-CAN, MED				1.5132	0.195	15
	NA-CAN, NA-ALG				1.0534	0.374	121
	SP, MED				3.7388	0.272	4
	SP, NA-ALG				1.5751	0.109	10
	MED, NA-ALG				1.8833	0.336	3

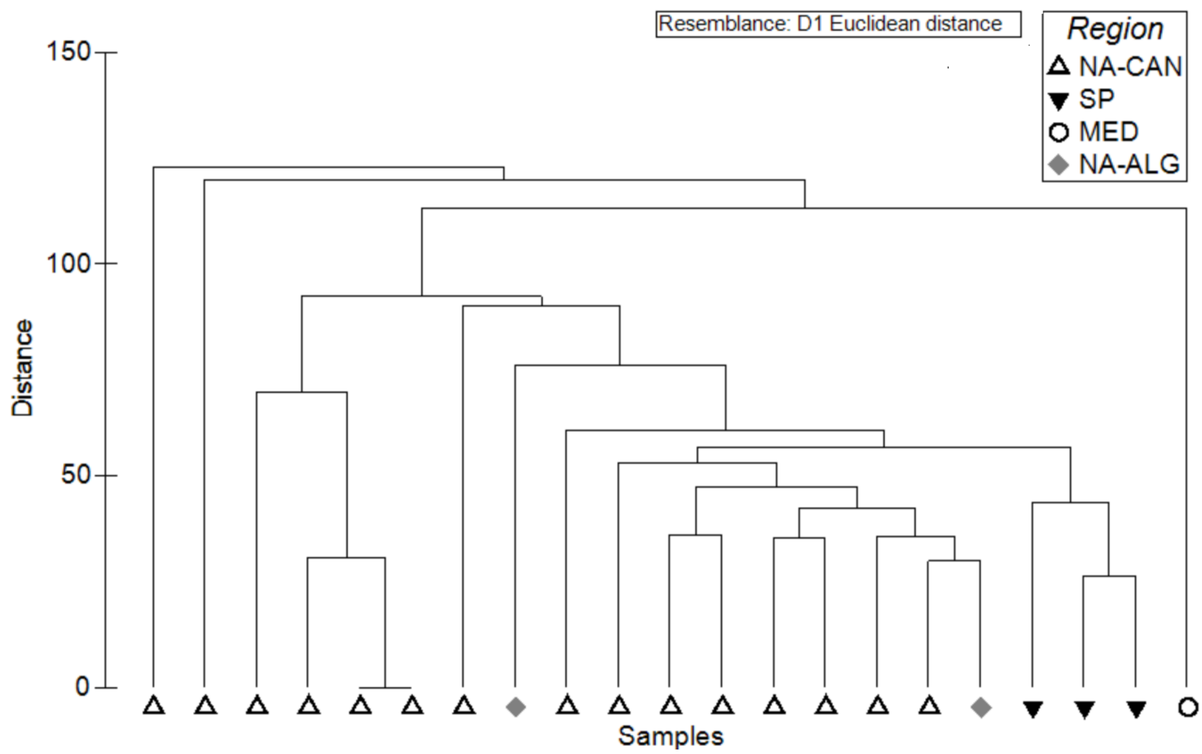

Figure 14. Grouping of sampling sites based on differences in composition of rafts. Regions: North Atlantic – Cantabrian (NA-CAN), North Atlantic – Algarve (NA-ALG), South Pacific (SP), Mediterranean (MED).

However, it needs to be considered that there was only one sampling site in the Mediterranean, which makes statistical analyses difficult. Statistically significant differences were detected between the South Pacific and the North Atlantic, more specifically the North Atlantic Cantabrian region (NA-CAN; Table 1, P1 and P2). Those were mainly due to the higher share of unidentified plastic fragments and the lower share of fishing gear among rafts found in the South Pacific than among rafts found in the North Atlantic (Figure 13; Table 2, SIMPER 1 and 2). Although the North Atlantic Algarve region (NA-ALG) is an aquaculture region, just like the Mediterranean sampling region, a higher similarity can be observed between NA-ALG and NA-CAN, than between NA-ALG and MED (Figures 13 and 14).

Table 2. Results of similarity percentage (SIMPER) analysis, showing the contribution of the most frequent item categories to the differences in raft composition between selected seas and regions. Item categories: PL24 = unidentified fragments and objects, PL17 = fishing gear, PL02 = bottles < 2L, PL14 = plastic buoys, PL19 = ropes, RB02 = rubber footwear, RB08 = rubber, not identified fragments and items. SD = standard deviation.

SIMPER 1	Average squared distance = 7691.69					
Variable	**North Atlantic** Average value	**South Pacific** Average value	Average squared distance	Squared distance / SD	Contribution [%]	Cumulative contribution [%]
PL24	23.1	75.5	3.43E3	1.14	44.6	44.6
PL17	28.1	4.17	1.71E3	0.56	22.3	66.9
PL02	8.59	0	633	0.27	8.23	75.1
PL14	5.88	0	588	0.25	7.65	82.8
PL19	4.51	10.8	385	0.48	5.01	87.8
RB02	8.71	0	307	0.45	3.99	91.8
SIMPER 2	Average squared distance = 8184.27					
Variable	**NA - Cantabrian** Average value	**South Pacific** Average value	Average squared distance	Squared distance / SD	Contribution [%]	Cumulative contribution [%]
PL24	21.3	75.5	3.65E+03	1.18	44.6	44.6
PL17	30.6	4.17	1.92E+03	0.6	23.5	68
PL02	8.4	0	690	0.27	8.44	76.5
PL14	6.67	0	667	0.26	8.15	84.6
RB02	9.87	0	348	0.48	4.25	88.8
RB08	4	0	240	0.26	2.93	91.8

From this meta-analysis, it seems that effectively the diversity of rafts could be at least a partial explanation of the diversity of biota. On one hand, this may be due to rafting biota´s different preferences for different materials or items, as is pointed out in chapter 1,2 and 4 of this Thesis (e.g. Thiel and Gutow, 2005b; Li et al., 2016). On the other hand, the categories for anthropogenic litter used in this Thesis are not only based on litter material, but also on source activities (e.g. fishing-related items). Therefore, the source activities, releasing anthropogenic litter rafts, may also influence the attached communities' species composition. It is notable that the most frequent litter items used as rafts (with exception of unidentified objects and fragments) all stem from sea-based sources, like fishing or shellfish aquaculture. Therefore, it may make sense to focus future studies on sea-based sources and related items.

2. Invasive species

2.1. Overview of invasive taxa

One of the most important results of the present Thesis was the very high frequency of NIS among all identified species from anthropogenic marine litter rafts. As many as one third of the taxonomic units that were identified on the species level were non-native invaders in the respective sampling areas. All identified NIS belonged to only four families of four phyla: Serpulidae (annelida), balanidae (arthropoda), bugulidae (bryozoa) and ostreidae (mollusca; Figures 15 and 16). The highest share of invasive species was found among barnacles of the family Balanidae, with 4 of 5 identified species being non-native and invasive, followed by oysters of the family Ostreidae, with 3 out of 4 identified species being classified as NIS, and serpulid tubeworms, with 4 NIS out of 8 identified species. The families Serpulidae and Ostreidae also contained several cryptogenic taxonomic units, that could not be classified as NIS or native.

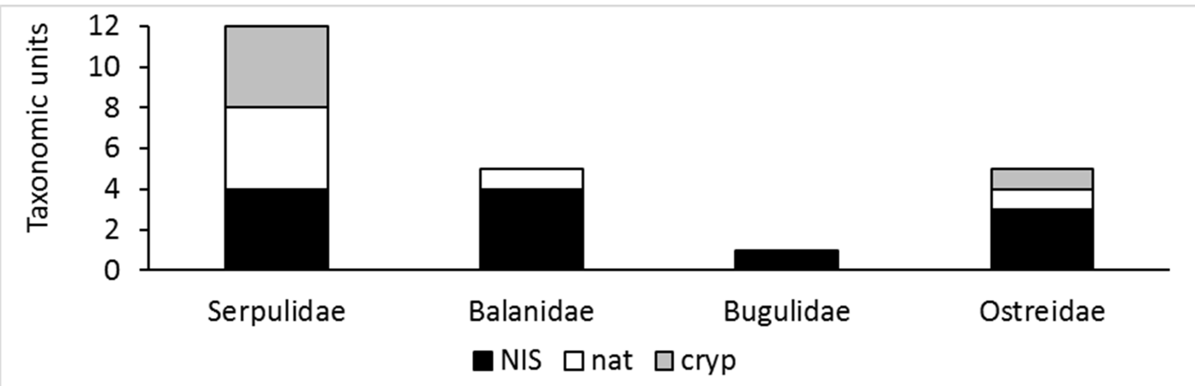

Figure 15. Taxonomic families containing non-native invasive species in the present Thesis.

On the phylum level arthropods were the most diverse group, containing similar shares of NIS, native species, and cosmopolitans. Annelids, bryozoans, echinoderms and molluscs had a high share of cryptogenic TU, meaning that the actual share of NIS might be higher than what is shown here (Figure 16).

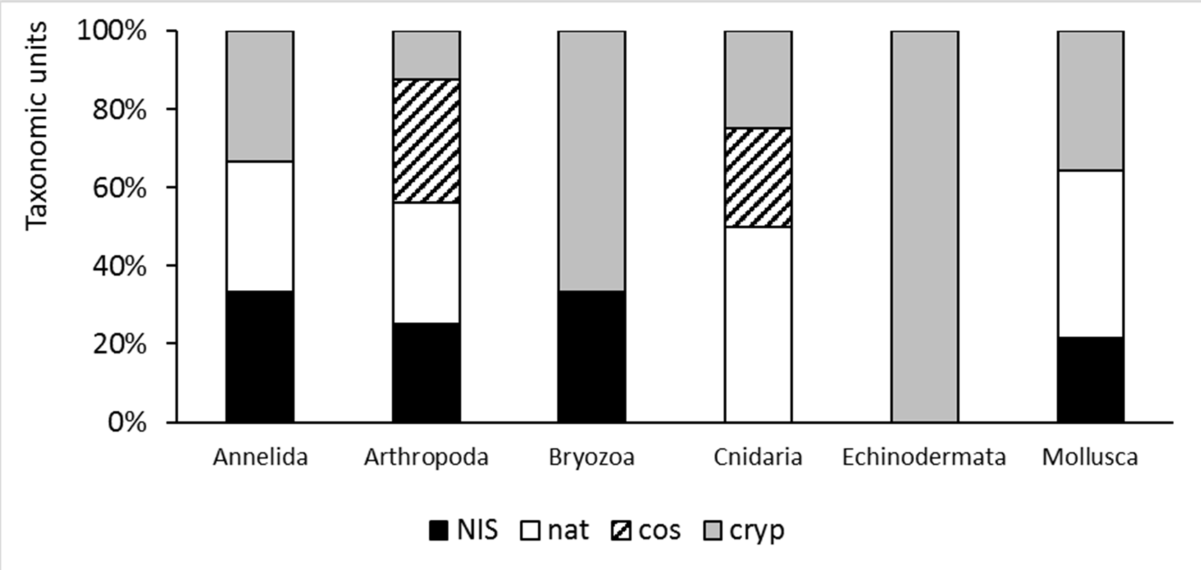

Figure 16. Percentage of non-native invasive species (NIS), native species (nat), cosmopolitan species (cos), and cryptogenic species (cryp) in the phyla found attached to rafts in this Thesis.

The four families contributing NIS detected in this study are known to contain several NIS with high impacts. Balanids are successful invaders. Of all non-native and cryptogenic barnacle species mentioned in a review by Carlton et al. (2011), 75% are balanids. Similarly, Torres et al. (2012) report that globally 69% of invasive barnacle species are balanids. The species found here are global invaders and are common in the respective sampling regions (North Atlantic coasts, Mediterranean; Torres et al., 2012; Gallagher et al., 2015; Ulman et al., 2017). *A. modestus* has been found on a variety of litter items on several beaches and in two sampling regions during this study. This invader has been recorded on stranded plastics before (Barnes and Milner, 2005) and the new findings discussed here show that rafting may be a mechanism for range-expansion of this species.

The polychaete family Serpulidae contains a variety of aggressive invasive species (Zenetos et al., 2005). *Hydroides sanctaecrucis* is known as an aggressive fouling pest in aquaculture facilities, which makes its dispersal by lost aquaculture gear quite likely (Lewis et al., 2006; Stafford and Willan, 2007). The fact that several individuals were found on different items of floating litter demonstrates the risk of this species' dispersal via rafting items, particularly on aquaculture-related litter.

The Bugulidae are a widespread family in the North Atlantic with several invasive fouling species (Ryland et al., 2011; Ramalhosa et al., 2017). *Bugula neritina* has been found along European Atlantic coasts and seems to be spreading northwards (Ryland et al., 2011). The family Ostreidae contains several invasive species which are cultured for human consumption.

The high numbers and percentages of NIS among rafting species on anthropogenic marine litter prove what has been repeatedly suggested by other authors: Anthropogenic marine litter is indeed a vector of transport for non-native invasive species. The results of the present Thesis suggest that it is much more common than previously thought, although its frequency seems to differ between geographic areas.

2.2. NIS by region and rafts

Comparing the geographic regions sampled during this Thesis, the two North Atlantic regions had the highest shares of NIS (Cantabrian: 25%; Algarve: 20%), followed by the Mediterranean (15%; Figure 17). No NIS were identified from the South Pacific; all TU identified there were native or cosmopolitan species. In the Cantabrian (North Atlantic) most TU were native or cosmopolitan species, while in the other three geographic regions most were cryptogenic.

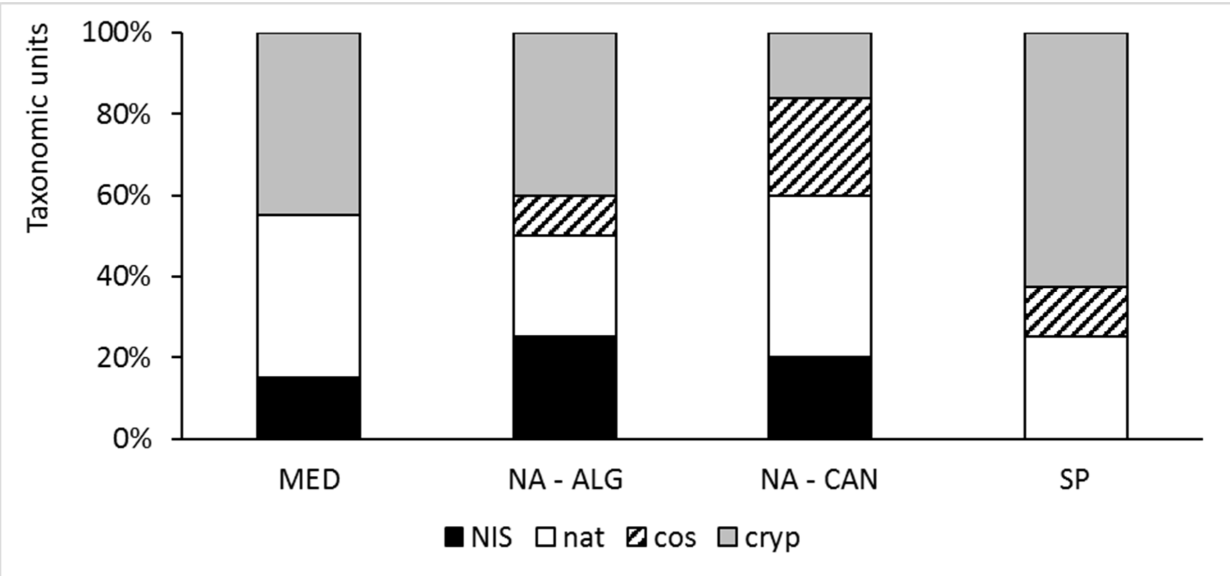

Figure 17. Percentage of non-native invasive species (NIS), native species (nat), cosmopolitan species (cos), and cryptogenic species (cryp) by geographic region.

The identified NIS were found on stranded anthropogenic litter in only one sampling region each, with one exception: *Austrominius modestus* was present on stranded plastics in large numbers in both North Atlantic regions (Algarve and Cantabrian, Table 3; Table S1). In the Algarve region 5 NIS were detected on the two beaches sampled, and in the Mediterranean region 3 NIS were detected on the only beach sampled. In contrast, in the Cantabrian region, where a total of 15 beaches was sampled, a total of 5 NIS were detected on only three beaches: Bayas, Penarronda and Figueras (Table 3).

Table 3. Non-native invasive species (NIS) per sea, region, beach and item category, and number of rafts found with the respective species attached. NA = North Atlantic, MED = Mediterranean, SP = South Pacific, ALG = Algarve, CAN = Cantabrian. For explanation of item categories see Table S2.

NIS	Sea	Region	Beach	Item category	Nr of rafts
Amphibalanus amphitrite	NA	ALG	Faro	PL02	2
				PL24	1
Austrominius modestus	NA	CAN	Bayas	PL24	1
			Penarronda	PL24	1
			Figueras	RB08	3
		ALG	Faro	WD04	1
				PL02	2
				PL24	2
Balanus trigonus	MED	MED	Lido	PL14	1
Bugula neritina	NA	ALG	Faro	PL02	1
Hesperibalanus fallax	NA	ALG	Faro	PL17	2
			Sagres	PL19	1
Hydroides elegans	MED	MED	Lido	PL15	8
Hydroides sanctaecrucis	MED	MED	Lido	PL14	1
				PL15	8
Magallana angulata	NA	ALG	Faro	PL02	1
Magallana gigas	NA	CAN	Figueras	RB08	3
				RB02	1
			Penarronda	PL24	1
Neodexiospira sp	NA	CAN	Figueras	RB08	1
Ostrea stentina	NA	CAN	Figueras	RB08	1
Serpula columbiana	NA	CAN	Penarronda	PL17	1

It is interesting that no NIS were found in the South Pacific region and corroborates what Carlton et al. (2011) had pointed out before with respect to barnacle invasions: *„Striking … are the few barnacle invasions that have occurred on the Pacific coast of South America and … these species (A. improvisus, A. amphitrite and A. reticulatus) are reported only from* *northernmost locations (Ecuador, Colombia, and Peru)."* In general, marine invasions along the South Pacific Chilean coast seem to be about one magnitude lower than in the northern hemisphere. Moreover, there are no reports of aggressive invaders along the South-eastern Pacific. The suggested reasons are the region's oceanographic characteristics, biotic resistance, and the relatively low level of stress along the coasts. The main sources of NIS introductions along the Chilean coast are aquaculture and maritime transport (Castilla and Neill, 2009). However, maritime transport, which is crucial for NIS introductions is much less frequent there than for example in Europe or Asia and is scarce on remote Rapa Nui (Easter Island), where the marine fauna is regarded as depauperate with a relatively high level of endemism and where there are no reports of NIS (Boyko, 2003; Figure 18). This may be another explanation for the absence of NIS from Rapa Nui. As rafts and attached biota are moved with oceanic surface currents, it seems that they are vectors of introduction or range expansion within a current system, even over trans-oceanic distances, but usually do not travel between current systems or oceans.

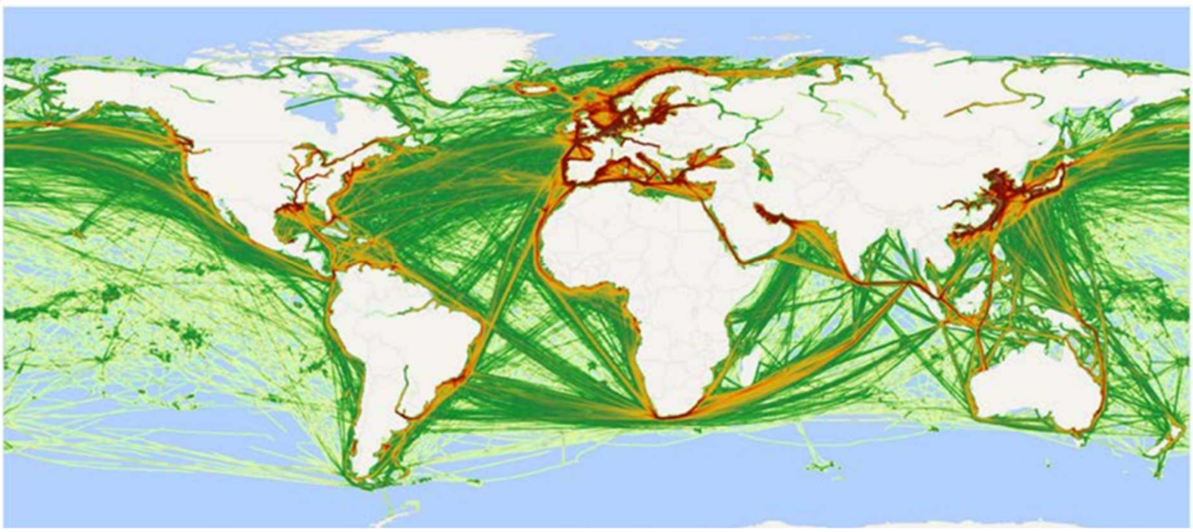

Figure 18. Global vessel traffic density in September 2014. Source: Wu et al., 2017.

Non-native invasive species were detected on 24 of the 173 rafts (14%) found during the samplings. They stemmed from 3 geographic regions (NA-CAN, NA-ALG and MED) and belonged to 9 UNEP categories (Figure 19, Table 4). Litter category PL15 (mussel bags) contained by far the highest number of rafts carrying NIS, followed by PL24 (unidentified fragments and objects), PL17 (fishing gear) and RB08 (rubber: unidentified fragments and objects, Figure 19, Table 4). Only PL17 and PL24 contained NIS rafts in more than one geographic region (NA-CAN and NA-ALG). In terms of frequencies of NIS rafts among all rafts, PL15, RB08 and WD04 (processed timber) had the highest percentages, followed by PL14 (plastic buoys, Table 4). However, except for PL15, these categories had very low absolute numbers.

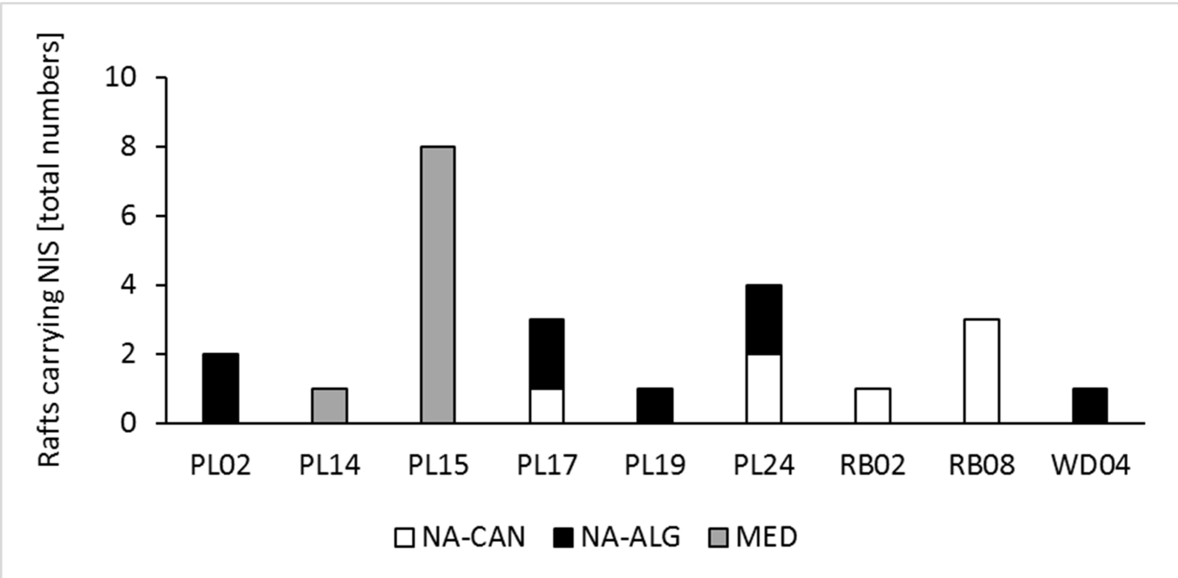

Figure 19. Rafts carrying NIS by litter category and geographic region. For explanation of item categories see Table S2.

Table 4. Number of all rafts and number of rafts carrying non-native invasive species (NIS) per item category and sampling region. NA = North Atlantic, CAN = Cantabrian Sea, ALG = Algarve, MED = Mediterranean Sea, SP = South Pacific. For explanation of item categories see Table S2.

	PL02		PL14		PL15		PL17		PL19		PL24		RB02		RB08		WD04	
REGION	**all**	**NIS**	**all**	**NIS**	**all**	**NIS**	**all**	**NIS**	**all**	**NIS**	**all**	**NIS**	**all**	**NIS**	**all**	**NIS**	**all**	**NIS**
NA-CAN	7	0	1	0	0	0	22	1	0	0	26	2	8	1	3	3	0	0
NA-ALG	2	2	0	0	0	0	2	2	3	1	5	2	0	0	0	0	1	1
MED	0	0	1	1	8	8	0	0	0	0	0	0	0	0	0	0	0	0
SP	0	0	0	0	0	0	3	0	8	0	40	0	0	0	0	0	0	0
TOTAL	9	2	2	1	8	8	27	3	11	1	71	4	8	1	3	3	1	1
NIS RAFTS [%]		22		50		100		11		9		6		13		100		100

In terms of biota diversity, PL02 (bottles < 2L) and RB08 (rubber: unidentified fragments and objects) were the most important litter categories, carrying a total of four different NIS each, followed by PL24 (plastic: unidentified fragments and objects), carrying three different NIS (Figure 20).

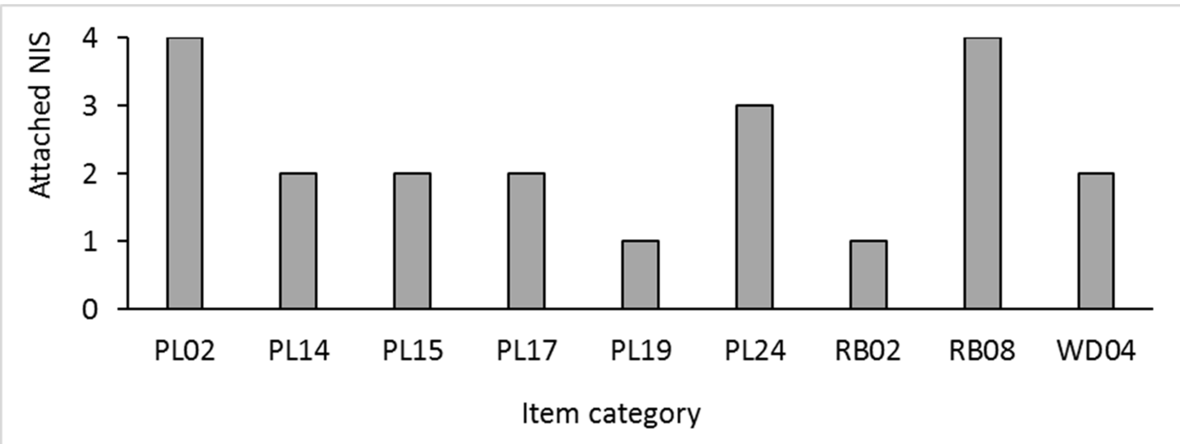

Figure 20. Number of attached non-native invasive species per item category. For explanation of item categories see Table S2.

Here again it is interesting that rafts of the categories PL02 and RB08 were only found on one beach each (Faro and Figueiras; Table 3), both of which are directly related to aquaculture activities. This shows once more, that more studies are needed to distinguish between the effects of an item’s material and source activity on the attached biota.

Conclusiones / Conclusions

CONCLUSIONS

1) Marine anthropogenic litter items, particularly plastics, are common vectors for the transport of attached biota, amongst them non-native and/or invasive species, to new habitats. Transport by rafting on anthropogenic marine litter is not an exception, but a frequent and ubiquitous global phenomenon. Depending on the geographic region, a high proportion of rafting biota can be non-native and invasive species (NIS). The number of rafting species (including NIS) is higher than previously known and still rising with new studies.

2) On a global scale, NIS rafting is more common in the North Atlantic and Mediterranean, than in the Southeastern Pacific environment, probably due to the much lower occurrence of NIS along Southeastern Pacific coasts. The species composition of rafting fauna differs between geographic regions/oceans. It can therefore be assumed that rafting on anthropogenic marine litter is a vector of species dispersal and introduction within a current system or an ocean, but not usually across oceanic barriers, formed by currents. Therefore, the importance of this vector may be higher in oceanic areas where NIS have already been introduced by other vectors, such as vessel transport or aquaculture. A regional focus of future studies is therefore suggested.

3) Source sites for rafting NIS are sites where a high frequency of NIS coincides with a high abundance of anthropogenic marine litter or artificial floating structures, which

may become detached. Aquaculture regions were identified as high-risk source areas of rafting NIS. Other sea-based activities, like fishing also provide a high share of rafts for attached biota. Measures should be implemented to avoid or at least reduce losses of anthropogenic litter and NIS from these activities and sites.

4) The species composition of the rafting fauna differs between rafts. This may be in part due to the item’s material and characteristics and in part due to its source region or the source activity releasing the item. Future studies should be designed to investigate both the effect of the items’ source and the effect of its physicochemical properties.

CONCLUSIONES

1) La basura marina con origen antropogénico, en particular los plásticos, son vectores para el transporte de biota adherida, entre ellos especies no autóctonas y/o invasoras, a nuevos hábitats. El *rafting* no es ocasional, o excepcional, sino un fenómeno global omnipresente y frecuente. Dependiendo de la región geográfica, una gran parte de la biota de *rafting* son NIS. El número de especies (incluyendo NIS) haciendo *rafting* es más alto de lo que se pensaba anteriormente y sigue aumentando con cada nuevo estudio.

2) El transporte de especies exóticas invasoras es más común en el Atlántico Norte y el Mediterráneo que en el entorno del Pacífico Sureste, probablemente debido a la mucho menor abundancia de especies exóticas invasoras a lo largo de las costas del Pacífico sudeste. La composición de especies de la fauna de *rafting* difiere entre regiones geográficas y océanos. Por lo tanto, se puede inferir que el *rafting* sobre la basura antropogénica es un vector de dispersión e introducción de especies dentro de un sistema de corrientes o un océano, pero de forma general no lo parece ser a través de las barreras oceánicas formadas por las corrientes. Por lo tanto, los futuros estudios deberían tener un enfoque regional.

3) Los sitios de origen para el *rafting* de especies exóticas invasoras son lugares donde coinciden una alta frecuencia de estas especies con una gran abundancia de

desechos marinos antropogénicos, o estructuras flotantes artificiales que pueden desprenderse. Las regiones de acuicultura fueron identificadas como áreas donantes de alto riesgo. Otras actividades marinas, como la pesca, también proporcionan una gran cantidad de *rafts* para la biota adherida. Se recomienda la implementación de medidas para evitar o al menos reducir las pérdidas de materiales procedentes de estas actividades.

4) La composición de especies de la fauna de *rafting* difiere entre las diferentes categorías de *rafts*. Esto puede deberse en parte al material y las características del elemento, y en parte a la región o actividad de origen en la que se libera el objeto flotante. Se recomienda el diseño de estudios encaminados a investigar tanto el efecto de la fuente de los objetos flotantes como el de sus propiedades fisicoquímicas sobre el tipo y abundancia de especies que pueden transportar.

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Supplementary Material

SUPPLEMENTARY MATERIAL

Table S1. List of all species found, compared by sampling regions. Bold: Non-native invasive species (NIS). MED = Mediterranean Sea, NA = North Atlantic Ocean, SP = South Pacific Ocean.

Phylum	Class	Order	Family	Species	Origin	MED Venice	NA Algarve	NA Cantabrian	SP Rapa Nui
Annelida	Polychaeta	Sabellida	Serpulidae	***Hydroides sanctaecrucis***	NIS	yes	no	no	no
				Hydroides elegans	NIS	yes	no	no	no
				Neodexiospira sp.	NIS	no	no	yes	no
				Sabellaria alveolata	nat	yes	no	no	no
				Serpula columbiana	NIS	no	no	yes	no
				Serpula vermicularis	nat	yes	no	no	no
				Spirobranchus taeniatus	nat	no	no	yes	no
				Spirobranchus triqueter	nat	yes	yes	yes	no
Arthropoda	Insecta	Hemiptera	Gerridae	*Halobates sericeus*	nat	no	no	no	yes
	Malacostraca	Amphipoda	Caprellidae	*Caprella andreae*	cos	no	no	yes	no
	Maxillopoda	Sessilia	Balanidae	***Austrominius modestus***	NIS	no	yes	yes	no
				Amphibalanus amphitrite	NIS	no	yes	no	no
				Balanus trigonus	NIS	yes	no	no	no
				Perforatus perforatus	nat	yes	no	yes	no

				Hesperibalanus fallax	NIS	no	yes	no	no
			Chthamalidae	*Chthamalus montagui*	nat	no	yes	yes	no
				Chthamalus stellatus	nat	no	no	yes	no
			Verrucidae	*Verruca stroemia*	nat	no	yes	yes	no
		Lepadiformes	Lepadidae	*Dosima fascicularis*	cos	no	no	yes	no
				Lepas anatifera	cos	no	yes	yes	yes
				Lepas anserifera	cos	no	no	yes	no
				Lepas pectinata	cos	no	yes	yes	no
Bryozoa	Gymnolaemata	Cheilostomatida	Membraniporidae	*Jellyella eburnea*	cryp	no	no	no	yes
			Bugulidae	***Bugula neritina***	cos - inv	no	yes	no	no
Cnidaria	Anthozoa	Scleractinia	Pocilloporidae	*Pocillopora (damicornis)*	nat	no	no	no	yes
	Hydrozoa	Anthoanthecata	Bougainvilliidae	*Bougainvillia muscus*	nat	no	no	yes	no
		Leptomedusae	Campanulariidae	*Obelia dichotoma*	cos	no	no	yes	no
Mollusca	Bivalvia	Adapedonta	Hiatellidae	*Hiatella arctica*	nat	no	yes	no	no
		Mytiloida	Mytilidae	*Mytilus edulis*	nat	yes	yes	yes	no
				Mytilus galloprovincialis	nat	yes	no	yes	no
		Ostreoida	Ostreidae	***Magallana angulata***	NIS	no	yes	no	no
				Magallana gigas	NIS	no	no	yes	no
				Ostrea edulis	nat - inv	yes	no	no	no
				Ostrea stentina	NIS	no	no	yes	no
		Pectinida	Anomiidae	*Anomia ephippium*	nat	yes	no	no	no
	Gastropoda	Trochida	Trochidae	*Steromphala umbilicalis*	nat	no	no	yes	no

Table S2. United Nations Environmental Programme (UNEP) litter categories and codes, used for the classification of rafts found in the studies of this Thesis. Source: Cheshire et al., 2009.

UNEP code	Material	Items
FP03	Foamed plastics	Foam buoys
FP05		Unidentified fragments and items
GC02	Glass	Bottles & jars
PL01	Plastics	Bottle caps & lids
PL02		Bottles < 2 L
PL03		Bottles, drums, jerrycans & buckets > 2 L
PL13		Baskets, crates & trays
PL14		Plastic buoys
PL15		Mesh bags (vegetable, oyster nets & mussel bags)
PL17		Fishing gear (lures, traps & pots)
PL19		Rope
PL21		Strapping
PL24		Unidentified fragments and items
RB02	Rubber	Footwear (flip flops)
RB08		Unidentified fragments and items
WD04	Wood	Processed timber and pallet crates
WD06		Unidentified fragments and items