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Alert calling in port areas: Marine litter as possible secondary dispersal vector for hitchhiking invasive species

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ABSTRACT

Floating plastic debris, such as bottles and fishing gear, is a shelter for different species in the oceans. Litter may therefore help the spread of non-indigenous species (NIS). Here we have challenged the idea of using the abundance of marine litter present in a zone to estimate the risk of NIS introduction. To test this, a targeted sampling of plastic bottles and fishing gear (ropes and nets) was performed along 22 beaches from the Cantabrian coast where ports have been reported as a source of biological invasions. All items with attached organisms were collected and recorded. Genetic barcoding was used to ascertain the species and identify NIS. In total 17 species attached to plastic bottles and fishing gears were identified. Three of them, found on the two types of items, are catalogued as invasive species: *Austrominius modestus*; *Magallana gigas*; and, *Amphibalanus amphitrite*. Prevalence and mean intensity of non-indigenous biota on plastic bottles and fishing gear were not significantly different. The abundance of barnacles in litter was significantly correlated with that found from ports in the same region. The results suggest that ropes are able to transport different marine organisms and NIS as plastic bottles do. Monitoring biota on marine litter could serve as an additional tool for NIS detection.

1. Introduction

Marine debris in oceans has been recognized as a major concern in marine conservation (Sutherland et al., 2010). Much more than an aesthetic problem (Mouat, Lozano, & Bateson, 2010), marine debris has severe consequences for both sea life and human health (Gregory, 2009). The types and quantity of anthropogenic debris vary around the world, but plastic items represent a higher proportion everywhere and pose concerns due to their long life and harmful effects on marine life while they degrade (e.g. Gall & Thompson, 2015). Moreover, recent studies found significant amounts of fouling organisms on plastic debris (e.g.Gündoğdu, Çevik, & Karaca, 2017), as well as microfouling communities (Maso, Fortuno, de Juan, & Demestre, 2016). Other types of marine debris are also a matter of concern. Abandoned, Lost or otherwise Discarded Fishing Gear (ALDFG) have considerably increased over the last 50 years (Macfadyen, Huntinton, & Cappell, 2009). ALDFG made of natural materials such hemp, cotton or straw, take about 3–14 months to completely degrade in the water column; however, traditional fishing gears are increasingly replaced by modern gears made of stronger and cheaper modern materials that last much longer in the ocean. Abandoned nets can be in the ocean for years, travel long distances (Kaiser, Bullimore, Newman, Lock, & Gilbert, 1996). Plastic ALDFG represent a hazard for fish stocks and the marine environment (Macfadyen et al., 2009); animals can get trapped inside, a phenomenon known as ghost fishing, and many end up dying of suffocation when they cannot distinguish between ALDFG and preys (Kaiser et al., 1996). However, in general, marine litter studies are more focused on plastic and microplastic effects than ALDFG which were sometimes neglected (e.g. Dias & Lovejoy, 2012; Galgani, Hanke, & Maes, 2015, Galgani et al., 2015).

Debris provide a new habitat for marine species adding new surfaces for colonization by organisms (Gündoğdu et al., 2017; Harrison, Sapp, Schratzberger, & Osborn, 2011). When a species is carried outside their native distribution and start proliferating in non-native areas, it may cause severe environmental and economic damage (Colautti & Macssa, 2004). In the new habitat, the introduced non-indigenous species (NIS) individuals may compete for natural resources and space leading to a decrease of endemic species or even to their extinction (Gurevitch & Padilla, 2004). Therefore, the increase of biological invasions challenges biodiversity conservation (Simberloff et al., 2013). Ordered by abundance, the more common organisms living on marine trash are bryozoans, barnacles, polychaete worms, hydroids and mollusks

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(Barnes, Galgani, Thompson, & Barlaz, 2009), and precisely all these groups contain invasive species. Thus, floating objects are considered potential vectors of invasive species (Rech, Borrell, & García-Vazquez, 2016for a review), despite some studies about fouling biota on marine debris not detecting any invasive species (e.g. Aliani & Molcard, 2003; Gündoğdu et al., 2017). Regardless of the magnitude of the problem, data about organisms carried by marine litter are relatively scarce and concentrate on a few regions of the world like for example the Mediterranean Sea (Gündoğdu et al., 2017; Maso, Fortuno, de Juan, & Demestre, 2016; Aliani & Molcard, 2003). One of the gaps is the North Iberian coast (south Bay of Biscay); an increasing occurrence of NIS associated with maritime traffic in ports therein has been reported (Pejovic et al., 2015; Devloo-Delva et al., 2016; Miralles et al., 2016a). Ports are well known gates of biological invasions (e.g.Molnar, Gamboa, Revenga, & Spalding, 2008; Ardura, Planes, & Garcia-Vazquez, 2015), but the contribution of other types of vectors to the dispersal and establishment of NIS in the south Bay of Biscay is still unknown. Floating marine litter moves with the currents and can be a vector for regional dispersal of invasive species arriving to ports. If it was true, marine litter nearby ports should be prioritized for monitoring and removal, since it could be the origin of regional expansion of exotics otherwise enclosed in the ports. A comparison of biota carried by marine litter and biota associated with ports can help to address this issue. One of the most accurate methods to identify this biota is DNA barcoding. DNA barcodes (short DNA sequences that enable species identification) are useful tools to accelerate species-level analysis of marine biodiversity and to facilitate conservation and biosecurity efforts (Bucklin, Steinke, & Blanco-Bercial, 2011).

This study focused on metazoans attached to plastic bottles and fishing gear, as representative of two different litter types. DNA barcodes were employed to unambiguously identify the different species attached to these items, including NIS. Taking into account that the main regional entry of biological invasions through maritime traffic is the international port of Aviles, where several invasive species have been recently described (Pejovic et al., 2015; Devloo-Delva et al., 2016; Miralles et al., 2016a; Miralles, Dopico, Devlo-Delva, & Garcia-Vazquez, 2016b), we expect that the NIS found in ports would be carried further offshore by marine litter. Furthermore, the south central Bay of Biscay is under the influence of the eastwards Iberian Poleward current (Gil, 2003). Thus, the main objective of our study is to test if frequency of each NIS on litter items would be higher in the areas surrounding the ports and eastwards. For that reason, our departure hypothesis is that marine debris (in this case plastic bottles and ropes) can be a NIS dispersal vector in port areas.

2. Material and methods

2.1. Study area and sampling design

The study area was the coast of Asturias, north Spain, within the Cantabrian Sea (Bay of Biscay). This area is under the influence of the eastwards Iberian Poleward current, occurring at the beginning of every winter (Gil, 2003). Cape Peñas in the center of the coast divides it in two distinct geological and ecological zones, being more influenced by cold upwelling in the western area (e.g.Muñoz-Colmenero, Turrero, Horreo, & Garcia-Vazquez, 2012). Sampling was carried out during high-coefficient low tides, during the end of winter 2016 (February and March) well after the pass of the Iberian Poleward current (Gil, 2003).

Twenty-two beaches (Fig. 1) within the coastal region were analysed. Eight main ports within the same region were surveyed for metazoans (Miralles et al., 2016a). Four sub-areas were considered taking into account the Cape Peñas as a boundary between colder western and warmer eastern zones: the west side of Cape Peñas (beaches Verdicio, Xagó, Zeluán, San Juan de Nieva, Salinas, Bayas); the west adjacent area (Figueras, Arnao, Peñarronda, Navia, Barayo, Otur, El Silencio beaches); the east side of Cape Peñas (Bañugues, Xivares, Arbeyal, Peñarrubia beaches); the adjacent east area (Rodiles, Santa Marina, Poo, Andrín beaches).

Each beach was visited once. All plastic bottles and ALDFG were collected from the vegetation line to the waterline. In the largest beaches (Bayas, Salinas, Xagó and San Lorenzo) sampling was restricted to five meters above and five below the high tide waterline, since most marine debris were located therein. Ropes and nets were classified as natural (e.g. straw, esparto) or not-natural (plastic). Size was recorded of plastic bottles. All the items with attached biota were taken to the laboratory for further analysis.

2.2. Species taxonomy and status assignment

All attached metazoans were collected and preserved in ethanol at the Laboratory of Genetics of Natural Resources, University of Oviedo. Lindner (1978) taxonomic guide was employed for morphological identification of all samples to the lower possible taxonomic level. World Register of Marine Species (WoRMS - World Register of Marine Species, 2015) was followed for taxonomic nomenclature. Invasive species status was checked from the Global Invasive Species Database (International Union for Nature Conservation, http://www.iucngisd.org/gisd/search.php, accessed on April 2017). Up to 15 individuals per morphotype (presumably species) and item were genetically analyzed.

2.3. Genetic identification

Small pieces of muscle tissues of approximately 2 mm^3 were taken for DNA extraction following Estoup, Largiader, Perrot, and Chourrout (1996) protocol. The E.Z.N.A. Mollusc DNA Kit was employed for molluscs and crustaceans according to manufacturer's directions. DNA samples were stored at 4 °C for further analysis.

DNA amplification of a fragment of the cytochrome oxidase I (COI) gene was performed with universal primers jgLCO1490 and jgHCO2198 (Geller, Meyer, Parker, & Hawk, 2013). These primers anneal on DNA from a wide group of marine invertebrates belonging to different taxa (Geller et al., 2013). Minor modifications were applied from the PCR amplification protocol proposed by Geller et al. (2013). PCR mixtures contained 1x Taq buffer, 2.5 mM MgCl2, 2.5 mM dNTPs, 1 µM primer jgLCO1490, 1 µM primer jgHCO2198, 0.03 µ/µM Taq polymerase (Promega), 0.2 mg/ml BSA and 4 µl of isolated DNA; summing up a final volume of 40 µl. PCR products were resolved on 2% agarose gels stained with SimplySafe to confirm amplification before sequencing.

Amplification of the 18S rDNA was done following Miralles et al. (2016a) on specimens with no amplification of COI gene. PCR products were sent to the company Macrogen (Macrogen, 2016) for DNA sequencing.

All sequences were edited with BIOEDIT (Hall, 1999) software and contrasted with Bold Systems (BOLDsystems, 2015) and nBLAST software in NBCI (Coordinators, 2013) with online public databases BOLD (Ratnasingham & Hebert, 2007) and GenBank (National Center of Biotechnology Information, 2015) respectively. The threshold for species assignation was at least 97% nucleotide identity, maximum E-value = 1e-100.

2.4. Statistical analysis

The data were treated following epidemiological approach, considering marine litter as vectors of NIS (potential nuisance species). Prevalence was the proportion of bottles or ALDFG carrying NIS over the total number of items of each type. Mean intensity was the mean number of NIS individuals over the items with NIS.

Shapiro-Wilk test was employed to check normality in the dataset. Non-parametric Kruskal-Wallis test served to compare biota medians when normality was not assumed. IBM SPSS Statistics v.23 software was employed for these tests. Prevalence was compared between groups using Fisher exact test also available in the previously cited software.