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Note

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

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ABSTRACT

The importance of mariculture areas for the dispersal of alien invasive species (AIS) on artificial floating items has recently been highlighted as a priority research need. Here we present the results of surveys in two important European shellfish culture areas that release rafting AIS, the Venetian lagoon and the Portuguese Algarve region. We found eight aquaculture-related non-native, invasive species attached to anthropogenic litter items mostly related to aquaculture: *Amphibalanus amphitrite*, *Austrominius modestus*, *Balanus trigonus*, *Hesperibalanus fallax*, *Hydroides elegans*, *Hydroides sanctaecrucis*, and *Magallana angulata*. These species are well-adapted to rafting on artificial surfaces and have a high potential to disperse via this vector. This is the first record of the notorious nuisance species *H. sanctaecrucis* both in the Mediterranean and Eastern Atlantic, as well as on floating litter. We also present the first records of *M. angulata*, *H. sanctaecrucis*, *Sabellaria alveolata*, *Mytilus edulis* and *Chthamalus montagui* on stranded anthropogenic litter.

1. Introduction

Aquaculture areas have been identified as high-risk source areas for the dispersal of invasive marine species on litter objects and the need for further research and management actions has been highlighted (Rech et al., 2016; Campbell et al., 2017). Aquaculture provides one third of the seafood globally and the industry is growing fast. The farming of aquatic plants and animals has led to the introduction of several invasive species, both intentionally in the case of farmed species, and accidentally in the case of associated ones (e.g. hitchhikers on mollusc shells; Naylor et al., 2001; Lee and Gordon, 2006; Mckindsey et al., 2007; Davidson et al., 2015; Cottier-Cook et al., 2016). 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).

Bivalve farming represents almost half of the European Union's (EU) aquaculture production (629,449 t live weight in 2015; European Commission, 2017). The main farmed shellfish species are the mussels *Mytilus edulis* and *Mytilus galloprovincialis* (European Commission, 2012), the oysters *Ostrea edulis* and *Magallana gigas* (European Commission, 2013a), and the clams *Ruditapes decussatus* and *Ruditapes philippinarum* (European Commission, 2013b). Several biologic pests have been introduced first as hitchhikers on farmed molluscs (e.g. the gastropods *Crepidula fornicata* and *Ocinebrellus inornatus*, or the ascidian *Ciona intestinalis*) which can not only harm native species and ecosystems, but also negatively impact the farmed species themselves (Naylor et al., 2001; Jensen and Knudsen, 2005; Mckindsey et al., 2007; Rius et al., 2011; Ryland et al., 2011; Fitridge et al., 2012). For example, 18 of the 33 species known to be associated with oyster culture are regarded as harmful (Molnar et al., 2008). The cultured non-indigenous species (NIS) themselves can also spread from aquaculture facilities and establish self-sustaining populations, thereby impacting the surrounding environment (Mckindsey et al., 2007).

The introduction of invasive species does not only occur with the movement of farmed species, but also with farm structures and equipment. Mariculture facilities are located in sheltered coastal spaces, especially estuaries, and provide many artificial hard substrates, thereby offering an optimal habitat for invasive species (Mckindsey et al., 2007; Tyrrell and Byers, 2007; Leonard et al., 2017). Examples of artificial substrates in these areas include shallow floating pontoons, which can host a variety of invasive species and have been shown to enhance invader's dominance (Glasby et al., 2007; Minchin, 2007; Dafforn et al., 2009); or mussel lines, which are often fouled by tunicates (Ashton et al., 2006; Lutz-Collins et al., 2009; Rius et al., 2011; James and Shears, 2016). The invasive amphipod *Caprella mutica*, for example, is common on aquaculture equipment and was found at 59% of sampled aquaculture sites along the Western coast of Scotland (Willis et al., 2004; Ashton et al., 2007).

The risk of aquaculture-related spreading of invasive species is not

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limited to the intentional transport of farmed species or farming equipment. Biota might also escape from aquaculture sites on detached gear and other floating litter (Campbell et al., 2017). So-termed "abandoned, lost or otherwise discarded fishing gear" (ALDFG), including aquaculture gear, is a global problem of high impact. While detailed data is available for ALDFG from fishing, estimates of gear losses from the farming sector are scarce (Macfadyen et al., 2009). Reportings of lost aquaculture gear include polystyrene floats (Cho, 2005; Fujieda and Sasaki, 2005; Hinojosa and Thiel, 2009; Hong et al., 2014; Liu et al., 2015), plastic rope (Campbell et al., 2017), food sacks from salmon farms (Hinojosa and Thiel, 2009) and buoys (Astudillo et al., 2009). In southern Chile, where mussel and salmon farms are among the most important world-wide, aquaculture gear was the main constituent of local marine litter (Hinojosa and Thiel, 2009). The Ahe atoll lagoon (French Polynesia), where many pearl oyster farms have been abandoned, is polluted by 20 types of farming-related derelict gear and litter (Andréfouët et al., 2014). Particularly high losses of gear and installations can occur due to extreme weather events, like the 2004 tsunami in Indonesia, which caused the loss of 88% and 100% of fish cages at two affected sites (Phillips and Budhiman, 2005).

Many rafting species have been found on floating ALDFG and aquaculture equipment (Kiessling et al., 2015). For example, Astudillo et al. (2009) found 116 species on detached aquaculture buoys off the Chilean coast. Several alien invasive species (AIS) have already been detected on ALDFG and aquaculture material, among them some molluscs which had crossed the North Atlantic ocean in bait jars and stranded on United Kingdom (UK) shores (Holmes et al., 2015). Although rafting may not be regarded as an important way of dispersal for non-native biota, the availability of rafting vectors has about doubled with the use of plastics (Barnes, 2002). In a recent review, Katsanevakis and Crocetta (2014) suggest that > 80% of invasive species in the Mediterranean might be able to raft on floating litter, while 13 of such species have already been found rafting on floating litter. It is now known that biota can not only attach to artificial objects, but also multiply and survive long time periods and distances on it (Winston et al., 1997; Hoeksema et al., 2012; Holmes et al., 2015).

Not all taxa and species however, are equally suited to rafting (Thiel and Gutow, 2005) and the availability of flotsam/jetsam, anthropogenic litter and AIS differs strongly between regions. Following the identification of aquaculture sites as potential donor areas for rafting invasive species (Hewitt et al., 2006; Cook et al., 2008; Arthur et al., 2009; Rech et al., 2016), the aim of the present work is to identify high-risk AIS, which can disperse from aquaculture regions via floating litter, using genetic barcoding for species ascertainment.

2. Material and methods

2.1. Sampling sites

Sampling was conducted between the beginning of March and mid-April of 2016 in two important European areas of shellfish aquaculture: the lagoon of Venice, Italy (Lido beach 45.41°N; 12.37°E) and in Portugal's Algarve region (Faro beach, 36.99°N; −7.97°E; Sagres beach 37.01°N, −8.93°E). The lagoon of Venice is a semi-enclosed environment with homogeneous environmental conditions. The open coast of the Algarve, however, comprises diverse environments. Therefore, two aquaculture sites were sampled here: The Faro installations are within the Ria Formosa national park wetlands, while the Sagres installations are situated in a rocky coastal area. Molluscs make up > 70% (18,000 t in 2013) of aquaculture production in the lagoon of Venice, making it the main production site of *R. philippinarum* in Europe. The regional production also includes the mussel *M. galloprovincialis*, cultured in long-line farms, as well as several fish and crustaceans, farmed extensively in embanked parts of the lagoon, called "valli" (Cautadella and Crosetti, 2012; MIPAAF, 2014). Clams are directly grown on the sea bottom and extracted with vibrating dredgers (Melaku Canu et al., 2011; MIPAAF, 2014). The Venice lagoon is a known hotspot and sink for alien species, but at the same time represents a source region for the secondary spread of alien species elsewhere (Occhipinti Ambrogi, 2000; Marchini et al., 2015). The invasive mussel *Xenostrobus securis* is only one of the alien species first reported from the Venice lagoon, before spreading to other regions in the Mediterranean and along Western European margins (Marchini et al., 2015). In their review, Marchini et al. (2015), listed 71 NIS, of which 55 are established in the Venice lagoon. Only three of the 71 species had been introduced intentionally for culture: the Manila clam *R. philippinarum*, the Pacific oyster *M. gigas*, and the rock oyster *Saccostrea glomerata* (Marchini et al., 2015).

The Algarve region is the most important aquaculture area in Portugal (4,620 t in 2016 = 41% of the total national production; Instituto Nacional de Estatística, 2017). Its main farming zones are Ria Formosa in the east and Ria de Alvor in the west. A 96% of Algarve aquaculture is for extensive rearing of molluscs, namely *R. philippinarum*, *M. galloprovincialis*, and the oysters *M. gigas* and *M. angulata* (Campos and Cachola, 2005; Ramalho et al., 2011). Extensive shellfish rearing is mostly conducted in muddy/sandy substrate in intertidal areas in aquaculture parks. There are also a few offshore longline farms of *M. galloprovincialis* and one of *Magallana* spp. (Ramalho et al., 2011). The Algarve region has the highest number of NIS on Portuguese mainland (Chainho et al., 2015).

2.2. Sampling strategy

Fouled stranded objects were collected from the whole vertical shore area at each sampling site, from the shoreline up to the natural or anthropogenic shore limit, as indicated by dunes, houses, streets, or similar structures. The sampling area was 8000 m^2, 7000 m^2, and 2800 m^2 at Lido, Faro and Sagres beach, respectively. Each fouled litter item was photographed, and the attached macroscopic fouling biota was identified based upon morphological characteristics. At each beach, the number of individuals of each taxon per type of item (e.g. mesh bag, plastic bottle) was counted. If there were > 50 individuals of a taxon, they were noted down as " > 50". A representative number of individuals of each taxon (≥5, except if there were less individuals of a taxon present) was detached from each litter item at each beach and stored in 96% ethanol for 12 months, before genetic analysis.

2.3. Data analysis

Litter items were grouped according to the categories and codes suggested by United Nations Environment Programme (UNEP; Cheshire et al., 2009) and assigned to one of two source categories: 1) Sea-based source, 2) Land-based/Unknown source. Simpson's Diversity Index and the composition of the attached macrobiotic community was calculated for each type of item at each sampling site and compared between sampling sites, sampling regions, and source categories, by a permutational analysis of variance (PERMANOVA; Anderson et al., 2008), based on Euclidean distances. Similarity percentage analysis (SIMPER) was conducted to quantify the contribution of each taxon to differences in community composition on items from different source categories. To avoid bias due to different levels of identification (some taxonomic groups were identified to the species level, while others could not be identified beyond the phylum or class level), all analyses were based on the number of individuals per phylum. The analyses were carried out with PRIMER 6 software (Clarke and Gorley, 2006). Results were regarded as statistically significant at a p-value of ≤0.05. Statistical comparison was not conducted between item categories, as several of them contained only one or very few items.

2.4. Genetic analysis and visual identification

Deoxyribonucleic acid (DNA) was extracted from a small piece of tissue (about 2 × 2 mm) using Chelex (Bio Rad BT Chelex 100 Resin),