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Assessment of the colonization and dispersal success of non-indigenous species introduced in recreational marinas along the estuarine gradient

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ABSTRACT

The introduction of non-indigenous species (NIS) is considered as one of the main causes associated with biodiversity loss. The number of NIS has increased significantly emphasizing the need to know and manage the processes of biological invasions. Recreational marinas are points of entry for potential colonizers and can act as stepping-stones for the spread of these species. The main objectives of this work were to assess the distribution patterns of the fouling communities within recreational marinas in the Tagus estuary and to evaluate NIS dispersion ability. The fouling communities were assessed at four different recreational marinas and neighbouring hard substrate areas along the estuarine gradient of the Tagus estuary. The native communities of those recreational marinas showed a spatial gradient consistent with the estuarine gradient, with higher similarity between marinas with higher marine influence. A total of 14 NIS were identified within the recreational marinas but none occurred at the marina with higher freshwater influence. Although NIS distribution pattern reflected, to some extent, the estuarine gradient, there was a lower heterogeneity between locations when compared to native communities, evidencing a greater tolerance of NIS to a wider range of environmental conditions. Nine of the NIS identified at recreational marinas were also found in the neighbouring areas, suggesting their dispersal ability. NIS that seem to be able to disperse outside the marinas have planktonic larval stages and higher environmental tolerance, which seems to have contributed to the successful spread. These results emphasize the importance of monitoring NIS occurrence in recreational marinas in order to have an early warning on the arrival of species with higher invasion risks and to prevent its dispersal to sensitive ecosystems.

1. Introduction

The number of non-indigenous species (NIS) occurring in estuaries and coastal areas has increased significantly along the last decades. These invaders have been identified as one of the major threats to marine biodiversity and can lead to severe ecological, economic and social impacts, emphasizing the need to assess and manage processes of biological invasions (Bax et al., 2003).

Shipping is one of the main introduction vectors in marine and brackish waters, since specimens or propagules may be transported by ballast water or fouling (Wasson et al., 2001; Seebens et al., 2013; Galil et al., 2014). First studies on NIS focused mainly on large commercial vessels since it was considered that recreational boats would not withstand large accumulations of fouling, mainly due to high cleaning frequencies, relatively high velocities and a low permanence (< 30 days) in a single port (Carlton and Hodder, 1995). However, more specific studies showed that recreational boats play an important role in NIS spread, especially at local level (*e.g.* Johnson and Carlton, 1996; Johnson et al., 2001; Bax et al., 2002; Cole et al., 2019; Martínez-Laiz et al 2019; Pelletier-Rousseau et al., 2019; Peters et al., 2019; Ulman et al., 2019). In fact, small vessels may carry diverse fouling communities, including animals and plants, and sessile and mobile *taxa* (Floerl and Inglis, 2005; Farrapeira et al., 2007; Davidson et al., 2010). The fouling epibenthic communities include sessile marine invertebrates (tunicates, bryozoans, serpulids, sponges and barnacles) and some motile animals (crabs, amphipods, chitons, among others) (Lord et al., 2015). These authors also demonstrated that large-scale NIS distribution patterns were promoted by commercial shipping, while

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recreational boats were the main responsible for the spread at local scale.

Recreational marinas are “sheltered islands” for marine organisms (Bax et al., 2002), playing a key role in the introduction of NIS. These artificial structures represent important points of entry for NIS (Glasby et al., 2007), since vessels transport these species from their natural distribution areas and marinas substrates provide a network of suitable habitats for their secondary spread (Ashton et al., 2006). Marinas usually have adequate conditions for the establishment and subsequent spread of fouling organisms by providing an extensive range of hard surfaces for fixation (breakwaters, pontoons, ropes and stakes) thus promoting the horizontal or vertical distribution of organisms both on intertidal and subtidal areas (Minchin et al., 2006). There are several examples of secondary spread provided by recreational navigation, which include the stalked sea squirt *Styela clava*, in marine ecosystems (Floerl and Inglis, 2005; Locke et al., 2007), and the zebra mussel *Dreissena polymorpha*, in freshwater ecosystems (Johnson et al., 2001).

According to Essink and Dekker (2002), communities with reduced biological diversity are more susceptible to invasions when compared to those with high biodiversity. However, after establishment, NIS secondary spread and colonization of the natural neighbouring ecosystems is not always successful because i) appropriate ecological niches may not be available for colonization; and ii) there is greater biotic resistance in these environments, such as potential predators and competitors.

Several factors influence the macroinvertebrate communities spatial distribution patterns such as currents, temperature and salinity, with a decrease in the communities richness with lower salinities (Piscart et al., 2005). In order to successfully establish NIS have to overcome several barriers such as geographical (i.e. transportation), environmental (e.g. hydrodynamism, temperature, salinity, pH, pollution levels) and biotic (*e.g.* relationships with native organisms) (Bulleri and Airoldi, 2005; Shucksmith and Shelmerdine, 2015). Since salinity is considered one of the most limiting factors for spatial distribution of aquatic organisms (Remane, 1934), with a lower species richness at upstream estuarine areas (with salinity values between 5 and 8), it is important to understand if that trend is also true for NIS or if there are specific estuarine regions more susceptible to invasions. In fact, previous studies, mainly in soft sediments, have recorded an increase in the number of NIS with increased salinity (Paavola et al., 2005; Lejeusne et al., 2014; Jimenez et al., 2018).

Portugal might have played a pioneer role in marine species introductions, related to the Portuguese intensive exploration voyages during the 15th and 16th centuries. The introduction of the Pacific oyster *Magallana gigas* seems to be associated to the Portuguese maritime exploration routes and several hitchhiker species might have been transported along with the oysters but, until very recently, little was known about the occurrence of NIS in Portugal. Some studies regarding ecological assessments of particular NIS had been conducted until 2015 (e.g. Cabral and Costa, 1999; Aquiloni et al., 2005; Sousa et al., 2008; Amat and Tempera, 2009; Sousa et al., 2009; Canning-Clode et al., 2013a,b; Ramalhosa et al., 2014; Vaz-Pinto et al., 2014), when the first systematic survey was carried out for coastal lagoons, estuaries and coastal areas in Portugal, including the Madeira and Azores archipelagos (Chainho et al., 2015). This work recorded a total of 133 NIS, included in 12 Phyla, with macroalgae (32%), arthropods (14%), tunicates (13%), molluscs (11%) and bryozoans (9%) as the most represented. The highest number of NIS was found in largest systems, such as the Tagus estuary (27). The number of NIS occurring in the Portuguese estuarine and coastal areas has been annually updated, with 166 NIS registered until 2017 (Chainho et al., 2018).

The increase in population and the expansion of coastal tourism has led to a surge of artificial structures on coastal regions giving response to the economic, residential and recreational demands of the populations (Bax et al., 2002), thus making alterations of natural intertidal and subtidal habitats, increasingly conspicuous (Chapman & Underwood, 2011). The Tagus estuary includes some of the main shipping and nautical activity harbours of Portugal. Several studies regarding invertebrate NIS have been conducted in this estuary, although assessing single species. The Chinese mitten crab *Eriocheir sinensis* was recorded for the first time by local fishermen in the late 1980’s and has a well-established population in the Tagus estuary (Cabral and Costa, 1999). The Manila clam, *Ruditapes phillipinarum*, was introduced in different estuarine systems in Portugal in the last decade and is currently the dominant bivalve species in some areas of the Tagus estuary, mainly in shallow bays with extensive intertidal areas (Chainho et al., 2014). Within the few studies developed in Portuguese recreational marinas (e.g. Ryland et al., 2011; Canning-Clode et al., 2013b; Chainho et al., 2015; Souto et al., 2018). Canning-Clode et al. (2013a) recorded 16 NIS at Quinta do Lorde recreational marina (Madeira, Portugal). However, little is known about communities that colonize recreational marinas, particularly NIS, and which estuarine areas could be the most favourable for their colonization.

Currently, there are several policy and regulatory instruments that address this problematic, but these are essentially focused on preventive measures through the management of pathways and vectors of introduction (Chainho et al., 2015), neglecting secondary spread along the coasts (Zabin et al., 2014). The Marine Strategy Framework Directive (MSFD) requires all European member states to achieve Good Environmental Status of marine ecosystems by 2020. Among the eleven descriptors required by the MSFD to assess the environmental status, a specific descriptor (D2) requires the assessment of NIS, addressing major aspects related to biological invasions, namely monitoring the spatial and temporal occurrence of NIS, their abundance and the assessment of the scale of the major pressures and impacts.

The implementation of this legal framework in Portugal requires a comprehensive baseline of NIS occurring at areas of higher human pressure, namely higher incidence of introduction vectors, such as commercial harbours (fouling and ballast water), recreational marinas (fouling) and aquaculture facilities. This study aimed to provide an integrated assessment of NIS introduced by fouling via recreational boating in one of the most invaded Portuguese estuarine systems.

The main objectives of this work were i) to assess the distribution patterns of the fouling communities (including NIS) found at recreational marinas along the estuarine gradient of the Tagus estuary, and ii) to evaluate the potential secondary spread ability of NIS established at the recreational marinas.

2. Materials and methods

2.1. Study area

The Tagus estuary (38° 40′N, 9° 8′W), located in the Portuguese central-west coast, is one of the largest estuaries in Europe and the largest in Portugal, with an approximate area of 325 km^2 (Fig. 1). It is a mesotidal estuary, partially stratified, where the effect of saline intrusion reaches Vila Franca de Xira, located nearly 40 Km upstream. The salinity stratification is more homogeneous in the summer, when the highest values are recorded downstream, at high tide and in the deeper layers (Costa, 1999). The Tagus estuary is an important harbour area, used as a major entry point to the Iberian Peninsula. There are four docks (Alcântara, Santo Amaro, Belém and Bom Sucesso) and four recreational marinas (Cascais, Oeiras, Parque das Nações and Vila Franca de Xira) located in the northern branch of this estuary. There are also several port facilities (containerized, fractional and roll-on/roll-off terminals, solid and liquid bulk terminals, and cruise terminals) with international traffic, serving as an important link between the Mediterranean, Europe, America and Africa.

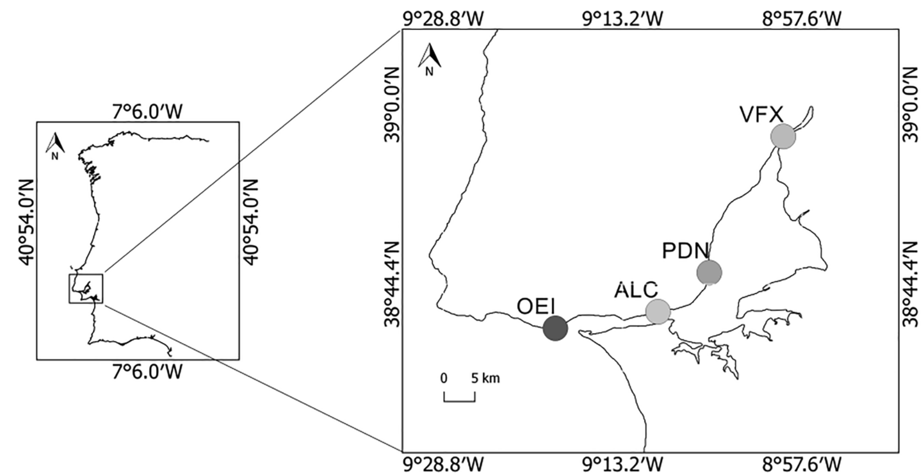

Fig. 1. Location of the sampling sites in the Tagus estuary (Coordinate system: WGS 84 - World Geodetic System, 1984). – Oeiras (OEI), Alcântara (ALC), Parque das Nações (PDN) and Vila Franca de Xira (VFX).

2.2. Sampling design

2.2.1. Recreational marinas

Four recreational marinas located along the Tagus estuarine gradient were selected for this study (Fig. 1): Oeiras (OEI), with a higher marine influence, Alcântara (ALC), located in the estuarine euhaline area, near a major commercial harbour area, Parque das Nações (PDN), located in the mesohaline area of the estuary, and Vila Franca de Xira (VFX), in the oligohaline area, with a higher freshwater influence. The Alcântara recreational marina is the oldest infrastructure, which was rehabilitated for recreational purposes in 1995/1996. The Parque das Nações was built during the 1998 Lisbon World Exposition, while Vila Franca de Xira and Oeiras are the most recent infrastructures, built in 2003 and 2005, respectively. Since cleaning activities influence the colonization by fouling communities, recreational marina's managers were asked about the cleaning frequency. All of them indicated that no frequent cleaning activities were conducted and these would take place only when floating piers would have been removed for maintenance.

Hard substrate communities were sampled in order to identify native and NIS invertebrates occurring in each of the four recreational marinas along the estuarine gradient of the Tagus estuary: Oeiras (April 2016); Alcântara (March 2016); Parque das Nações (June 2016); Vila Franca de Xira (October 2016). The sampling method consisted on scraping an area of 20 × 20 cm on the pontoons. PVC and concrete substrates were sampled randomly at each marina, to collect the colonizing subtidal fauna. A total of 24 samples were collected at each recreational marina.

2.2.2. Neighbouring areas

The neighbouring habitats of the recreational marinas were sampled during May 2017, to assess the NIS colonization success of these ecosystems. Areas located immediately upstream and downstream of the recreational marinas were selected, and samples of invertebrates were collected on the hard substrate intertidal areas, applying the same technique used within the recreational marinas. The number of samples collected was proportional to the availability of diverse substrates (i.e. stone blocks, rock platforms, pebbles, tires and other artificial structures). Three samples of the fouling communities were collected at Parque das Nações, four samples at Oeiras and five samples at Alcântara and Vila Franca de Xira.

2.3. Laboratory procedures

All samples were sorted and invertebrates retained in a 0.5 mm mesh size were identified to the lowest possible taxonomic level. All species were classified as NIS or native according to criteria defined by Chainho et al. (2015). In order to avoid an overestimation of the invasion rate the *taxa* that were not identified up to the species level were considered as native species. All invertebrate organisms were preserved in alcohol 70%, except tunicates, which were preserved in formaldehyde 10%.

2.4. Data analysis

Pearson's Chi-square tests for independence (χ^2) were conducted (one per marina) to evaluate the relationship between the number of species (proportion of native species and NIS) and the sampled sites (Inside and Outside areas), using Software R (R Core Team, 2013), considering a significance level of $p < 0.05$. This statistical test enables to understand if two crossed variables have no association (null hypothesis) or if the data show evidence of an association between the tested variables. Post-hoc tests were conducted to identify the sources of independency.

Spatial distribution patterns of invertebrate communities (native and non-indigenous) of the Tagus estuary were assessed based on presence/absence data since both colonial (*e.g.* bryozoans, sponges) and solitary organisms were considered. A PCO analysis (Principal Coordinate Ordination) was performed using a presence/absence matrix of all the fouling invertebrates recorded at the recreational marinas (Oeiras, Alcântara, Parque das Nações and Vila Franca de Xira) and in their neighbouring areas. Both cryptogenic species and *taxa* that have not been identified to the species level were considered as native to avoid the overestimation of the invasion rates and patterns. This ordination used Bray-Curtis similarity as a resemblance measure and was performed using the Primer 6 software (Clarke and Gorley, 2006). Pearson's rank correlations of the invertebrate species with the PCO axes were represented as vectors overlapping the first two axes of the PCO, to understand which *taxa* were more related to spatial patterns identified in the ordination. Only *taxa* with a Pearson's correlation coefficient higher than 0.5 were represented. Vectors length and direction represent respectively the strength and sign of the relationship of each species with the PCO axes. A second PCO was produced using only the NIS community matrix, to understand if spatial patterns were similar or distinct of the overall community. Major NIS drivers in the ordination were represented by overlapping vectors with a Pearson's correlation coefficient higher than 0.5.

3. Results

A total of 116 *taxa* of invertebrates were identified in this study (Annex I), including 46 Arthropoda, 21 Annelida, 18 Mollusca, 11 Chordata, 10 Bryozoa, 3 Echinodermata, 3 Porifera, 1 Nemertea, 1 Cnidaria, 1 Platyhelminthes, and 1 Sipuncula.

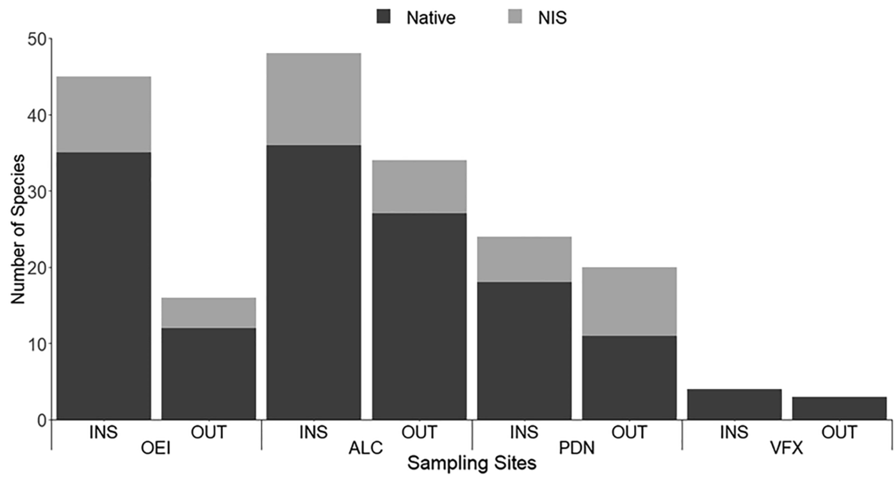

Fig. 2. Species richness of the fouling invertebrate community found inside the recreational marinas (INS) and in the respective neighbouring areas (OUT) at Oeiras (OEI), Alcântara (ALC), Parque das Nações (PDN) and Vila Franca de Xira (VFX).

A total of 93 *taxa* of different fouling invertebrates were identified at the recreational marinas, 15 of which were NIS. The highest number of native species was recorded at the Oeiras and Alcântara marinas (35 and 36, respectively), followed by Parque das Nações (18) and Vila Franca de Xira (4). Alcântara recreational marina presented the highest number of NIS, with 12 species recorded, followed by Oeiras (10) and Parque das Nações (6). Only native species were found at Vila Franca de Xira recreational marina (Fig. 2).

A total of 55 different *taxa* were recorded in the marinas neighbouring areas, 9 of which were NIS. The highest number of native fouling invertebrates was recorded at Alcântara (27), followed by Oeiras and Parque das Nações (11 and 12, respectively). The lowest number of native species (3) was found in the neighbouring areas of the recreational marina of Vila Franca de Xira. The highest number of NIS was recorded at Alcântara (9) and Parque das Nações (9) neighbouring areas, and only 4 NIS were identified at the Oeiras neighboring area, while no NIS were found at Vila Franca de Xira neighbouring areas (Fig. 2).

The number of native species was always higher within recreational marinas when compared to neighbouring areas. A decrease in native species richness was observed from downstream higher salinity (Oeiras and Alcântara) to upstream lower salinity areas (Parque das Nações and Vila Franca de Xira). This trend was not observed for NIS, since Parque das Nações neighbouring areas had a higher number of NIS than Oeiras outside areas. A higher number of NIS was also found at Parque das Nações neighbouring areas when compared to the inside area of the marina.

Pearson's Chi-square tests for independence showed that native invertebrate richness had a significant association with the sampling locations, since all comparisons were statistically significant ($X^2 = 65.73$; $p < 0.05$).

Sampling sites were ordered from the highest to lowest number of species recorded and three groups of locations were identified for native communities (Fig. 3): i) sampling sites with a higher species richness, namely inside the recreational marinas of Alcântara, Oeiras and Parque das Nações; ii) the outside areas of the previous recreational marinas; and iii) sampling sites with lower species richness. These results indicate differences in the number of species found inside and outside recreational marinas, with a higher probability of finding higher species richness inside the marinas.

Post-hoc tests were performed to identify in which sampling locations this association was significant (Fig. 3). Tests conducted on NIS community richness indicated that this attribute was independent of sampling locations ($X^2 = 5.25$; $p = 0.39$), except for Vila Franca de Xira, where no NIS were found.

The comparison between NIS found in recreational marinas (14) and in the neighbouring areas (9) indicated that 64% of the NIS had the potential ability to spread outside (Table 1.). Species with the highest success rate in colonizing the neighbouring areas were the amphipod *Caprella scaura,* the ascidian *Microcosmus squamiger* and the bryozoans *Tricellaria inopinata* and *Watersipora subtorquata*, since they were recorded at all outside areas of the recreational marinas where NIS were identified. *Watersipora subtorquata* occurred in all recreational marinas and respective neighbouring areas, while *C. scaura* was only recorded at the outside areas. Additionally, although *M. gigas* was identified in all recreational marinas, this NIS was not recorded in any of the neighbouring areas. Similarly, some of the other NIS observed inside recreational marinas, such as the ascidians *Styela clava, Styela plicata* and *Molgula manhanttensis*, were not found in the neighbouring areas. The bryozoan *Bugula neritina*, although recorded at all marinas, was only observed at the Parque das Nações neighbouring area.

Three distinct spatial groups were identified in the PCO using presence/absence of the fouling invertebrate species found at recreational marinas and their neighbouring areas (Fig. 4). Samples collected at Vila Franca de Xira were separated from all others along the first axis of the ordination, which explained 27.4% of the data variation, and were

Fig. 3. Post-hoc tests' results for native community samples. The segments connect sampling sites [inside the recreational marinas (INS) and in neighbouring areas (OUT) of Oeiras (OEI), Alcântara, Parque das Nações (PDN) and Vila Franca de Xira (VFX), where the native community's richness was not independent on the sampling location. Sampling sites are displayed according to the species richness (from the higher to the lower).

Table 1
Fouling invertebrate NIS registered inside the recreational marinas (INS) and respective neighbouring areas (OUT).

	Oeiras		Alcântara		Parque das Nações	
	INS	OUT	INS	OUT	INS	OUT
Mollusca						
Chaetopleura angulata	–	–	x	–	–	x
Magallana gigas	x	–	x	–	x	–
Arthropoda						
Amphibalanus amphitrite	x	–	x	x	–	x
Austrominius modestus	x	–	x	x	x	x
Balanus trigonus	x	–	x	x	–	x
Caprella scaura	–	x	–	x	–	x
Palaemon macrodactylus	–	–	–	–	x	–
Rithropanopeus harrisii	x	–	–	–	–	–
Bryozoa						
Bugula neritina	x	–	x	–	x	x
Tricellaria inopinata	x	x	x	x	–	x
Watersipora subtorquata	x	x	x	x	x	x
Chordata						
Microcosmus squamiger	x	x	x	x	–	x
Molgula manhattensis	–	–	x	–	x	–
Styela clava	–	–	x	–	–	–
Styela plicata	x	–	x	–	–	–
Number of NIS	**10**	**4**	**12**	**7**	**6**	**9**

mostly associated with the occurrence of the amphipod *Corophium orientale*. A second group included samples collected in Oeiras and Alcântara, which separated from Parque das Nações along the second axis, which explained 16.6% of the variation, and were characterised by higher occurrences of *W. subtorquata* and the bivalve *Mytilus galloprovincialis,* while samples at Parque das Nações were associated to the bryozoan *Conopeum seurati*, Porifera and *M. gigas.* The fouling invertebrate communities recorded at the neighbouring areas of Alcântara and Vila Franca de Xira seem to be more similar to those found within the respective marinas. On the contrary, the communities in Oeiras and Parque das Nações neighbouring areas appear to be more distinct of those found within the respective marinas.

The second PCO based only on the NIS fouling invertebrates (Fig. 5) showed a separation of Parque das Nações recreational marina along the first axis, which explained 43.0% of the variation, associated to the occurrence *M. gigas* and *M. manhattensis*. The cirriped *Amphibalanus amphitrite*, *W. subtorquata* and *B. neritina* were mostly associated to the recreational marinas of Oeiras and Alcântara. A separation between NIS communities found inside and outside the recreational marinas is

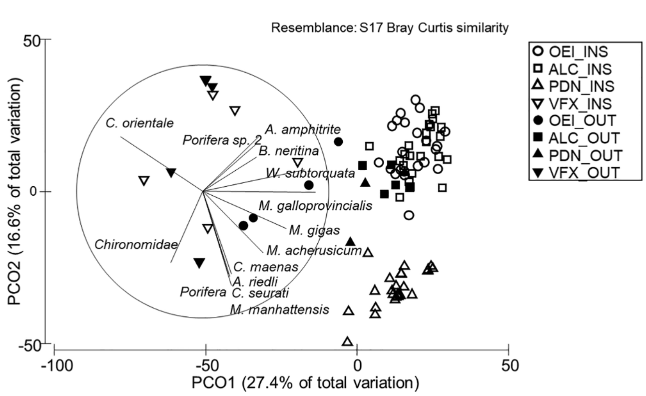

Fig. 4. Principal Coordinate Ordination (PCO) of the fouling invertebrate communities (presence/absence) recorded at the Oeiras (OEI), Alcântara (ALC), Parque das Nações (PDN) and Vila Franca de Xira (VFX) recreational marinas (INS) and respective neighbouring areas (OUT)). Vectors represent correlations between species and the first axes (PCO1 and PCO2) of the ordination (Pearson correlation < 0.5).

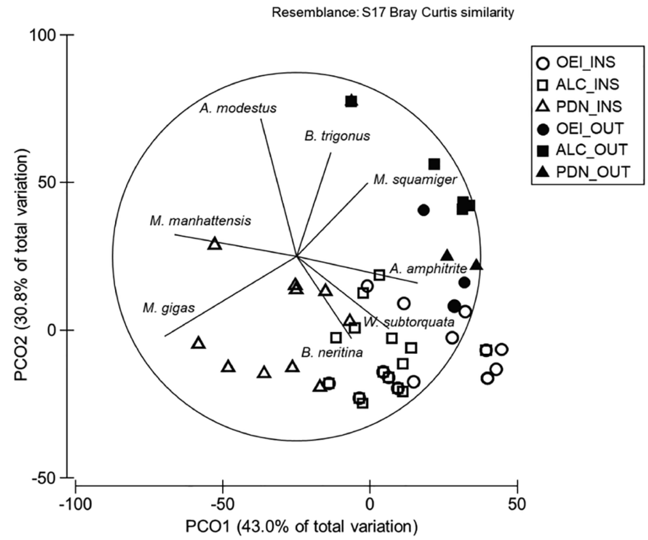

Fig. 5. Principal Coordinate Ordination (PCO) of the fouling NIS communities (presence/absence) recorded at the Oeiras (OEI), Alcântara (ALC), Parque das Nações (PDN) and Vila Franca de Xira (VFX) recreational marinas (INS) and respective neighbouring areas (OUT). Vectors represent correlations between species and the first axes (PCO1 and PCO2) of the ordination (Pearson correlation < 0.5).

observed along the second axis of the ordination, which explained 30.8% of the variation, although no segregation was observed between different neighbouring areas. The occurrence of the cirriped *Balanus trigonus* and *M. squamiger* appeared to be associated to all neighbouring areas.

4. Discussion

Shipping has been identified as the major vector of introduction of marine NIS (Ruiz et al., 2000) and is the dominant potential pathway for NIS introduction in the Tagus estuary (Chainho et al., 2015), since several commercial harbours and recreational marinas are located along the estuarine gradient. The results obtained in this study performed at four different recreational marinas indicated the occurrence of 15 NIS, which represent 12% of the total number of fouling invertebrate species identified. Two of these NIS, the crustaceans *Rhithropanopeus harrisii* and *Palaemon macrodactylus*, were single specimens collected at the recreational marinas of Oeiras and Parque das Nações, respectively. *R. harrisii* has been previously found in the Tagus estuary in mobile substrates (Projecto-Garcia et al., 2010; unpublished data). But this was the first record of *P. macrodactylus* in the Tagus estuary, since it had previously been recorded only in the Guadiana estuary (Chícharo et al., 2009). The ratio between NIS and native species found in this study was very similar to what was observed in other recreational marinas in the Azores islands, Portugal (Vaz-Pinto et al., 2014), Ligurian Sea and Sardinian Sea, Italy (Ferrario et al., 2017) and Bay of Brest, France (Kenworthy et al., 2018). Nevertheless, much higher invasion rates were found in the San Francisco Bay, within a comparative study on invasions in soft and hard substrates (Jimenez et al., 2018) that registered a very high representativeness of NIS over native species (73%-85%). At this bay has the highest NIS richness was documented NIS richness of any estuary in the world (Jimenez & Ruiz, 2016), representing on average 73%–85% and 46%-60% of total richness per sample in hard bottom and soft sediment communities, respectively (Jimenez & Ruiz, 2016).

Coastal lagoons and estuaries have been indicated as major hotspots of invasions, not only due to the occurrence of pathways that facilitate the introduction and secondary dispersal of NIS, but also because natural and anthropogenic disturbance that characterise such environments are colonized by low diversity and low-competition biota that can easily be replaced by opportunistic species, such as NIS (Occhipinti-Ambrogi & Savini 2003). The invasion rates identified in the Tagus estuary corroborate this tendency, since it is one of the largest European estuarine systems, one of the major commercial Portuguese harbours, with the highest number of recreational marinas.

4.1. Salinity gradient vs susceptibility to invasions

Local dispersion of NIS beyond the introduction areas depends on NIS life history characteristics and on the availability of internal spread vectors (e.g. local boating routes) but also on the occurrence of preventive/favourable environmental gradients. Salinity is considered as an important limiting factor for most aquatic species over other ecological factors. In transitional systems, such as estuaries, salinity determines most of the species range limits and colonization potential (Remane & Schlieper, 1971).

An overall increase in the number of NIS and native species was observed towards higher salinity areas in the Tagus estuary. These results are similar to previous studies on macroinvertebrate communities' spatial distribution patterns in estuarine systems (e.g. Ysebaert et al., 2003; Piscart et al., 2005; Chainho et al., 2006; Jimenez et al., 2018) and with the findings of Foster et al. (2016) that recorded a lower number of NIS in recreational marinas near freshwater sources in England. This is related with to higher salinity fluctuations in the oligohaline and mesohaline areas of estuarine systems, which increase physiological stress and induce a reduction in the number of species (Sanders et al., 1965). The reduced number of species recorded in Vila Franca de Xira and the lower diversity found in the Parque das Nações recreational marina show that pattern. This might be related to the so called "paradox of brackish water", initially proposed by Remane (1934), since the smallest number of species was found in areas with a higher freshwater influence. Seasonality might also have contributed to the results since sampling had to be carried out in different times of the year thus influencing the occurrence of species due to variations inherent to their life cycle.

NIS dispersal at local scale was also assessed in this study, by comparing the occurrence of NIS inside recreational marinas and in the neighbouring areas. Although no significant differences were found between inside and outside the recreational marinas, a higher number of NIS was registered inside the marinas, as in previous studies (Glasby and Connell, 2001; Paulay et al., 2002). Most NIS established inside recreational marinas occurred also in the neighbouring areas (60%), similarly to what was observed in San Francisco Bay, USA (Jimenez et al. 2018). Similar spatial patterns were observed for native and NIS species, with a decrease on species richness from higher to lower salinity areas, but NIS community showed a higher homogeneity between marinas than the overall fouling invertebrate communities. This may indicate higher tolerances to environmental conditions along the estuarine gradient, although the occurrence of NIS seemed to be constrained by very low salinities, since no NIS were recorded in Vila Franca de Xira.

Only four NIS were able to spread to all marinas neighbouring areas: *C. scaura, T. inopinata, W. subtorquata* and *M. squamiger*. All these species have high tolerances to wide environmental conditions, namely temperature and salinity (e.g. Dyrynda et al., 2000; Derungs, 2016), facilitating the colonization of different areas. Since dispersal occurs during the planktonic larval stage for most marine invertebrates with the duration of that phase and physico-chemical factors as relevant factors affecting the dispersal ability of NIS (Levin, 2006). Of all NIS that were able to disperse all the studied marinas, only *W. subtorquata* has a short life cycle and rapid fixation to the substrate, reducing their dispersion potential (Derungs, 2016).

Local spread might be constrained by small scale environmental gradients confining introduced species to the arrival area and preventing further secondary spread over the surrounding ecosystems. Although a higher propagule pressure may lead to greater invasion success in marine environments, this relationship is not consistent across habitat types, with higher establishment in artificial substrates (e.g. dock floats) than in natural habitats (Simkanin et al., 2017). The lack of benthic predators is indicated as a possible reason for a higher NIS establishment success in marinas (Simkanin et al., 2017), but small-scale gradients occurring between these artificial areas and adjacent natural environments have been overlooked. Recent results (unpublished data) suggest that significant vertical (i.e. stratification) and crosswise gradients (*e.g.* temperature, salinity, current velocity) might occur between confined marinas and surrounding ecosystems and could act as barriers to prevent colonization outside these structures.

Several studies have compared fouling communities inside and outside the marinas at different depths using artificial plates showing that NIS were disproportionately more numerous on shallow, moving plates (i.e. floating surfaces), than on deeper plates and inside the marinas (Webb and Keough, 2000; Dafforn et al., 2009). Despite the importance of this type of studies to evaluate the capacity of NIS for colonizing new substrates (i.e. with no fouling), these might overestimate the invasion potential. Therefore, assessments conducted on real available habitats/conditions for colonization of NIS, such as this study, are crucial to evaluate real conditions and to provide better estimates of NIS impacts. Comparisons between communities occurring in artificial and natural habitats are important to understand impacts of biological invasions on the epibenthic communities of aquatic ecosystems (Bulleri and Chapman, 2004). Nevertheless, it is still not clear if the availability of artificial structures promotes the colonization by NIS (Mineur et al., 2012), although occurrence of a high diversity of NIS in anthropogenic structures (floats, stakes, cables and buoys) has been recorded in recreational marinas (e.g. Davidson et al., 2010; Murray et al., 2011). The spreading and establishment by NIS into natural ecosystems adjacent to recreational marinas has been observed (e.g. Bacchiocchi and Airoldi, 2003; Borsje et al., 2011; Ros et al., 2013), but it was not always successful due to the lack of available habitat or to the greater biotic resistance on those habitats (Simkanin et al., 2013). In the present study, a total of 9 NIS identified at the recreational marinas were also found at the neighbouring areas, thus suggesting the dispersal ability of 64% of these species in the Tagus estuary. The fact that some artificial structures, such as tires, have been sampled in the Alcântara and Parque das Nações neighbouring areas but were not available in the other recreational marinas may have contributed to the smaller number of NIS (4) found at the outside area of Oeiras since only natural substrates were sampled. Previous studies indicate that natural substrates are preferably colonized by native species and, therefore, there are less available niches for NIS (Tyrrell and Byers, 2007; Gestoso et al., 2017).

Although a higher diversity of native species was observed in the neighbouring areas with increasing salinities (Oeiras and Alcântara), the same pattern was not verified for the NIS community. Parque das Nações registered a higher NIS richness outside than within the recreational marina. Some species, such as the chiton *Chaetopleura angulata* and *A. amphitrite*, were found only in the neighbouring area of Parque das Nações. These species are very old introductions and have a very wide distribution in estuarine systems and might have been introduced in the Tagus estuary by other vectors (Chainho et al., 2015). Moreover, since samples in the marinas and neighbouring areas were collected in two consecutive years, some species' life cycle might have been influenced by interannual variations and, consequently, may have not been detected.

All NIS identified in the neighbouring areas also occurred in the recreational marinas, except for the *C. scaura*. The absence of this caprellid inside the recreational marinas may reflect the sampling occasion since this species has been recorded in several Portuguese recreational marinas, including in the Tagus estuary, namely at the Cascais marina (Ros et al., 2014; Chainho et al., 2015) and more recently at Alcântara and Parque das Nações marinas (unpublished data). Since this species has a population density peak in March (Prato et al., 2013) it might have been overlooked because of lower abundances during the sampling periods. The higher number of NIS in the estuarine mesotidal area also reinforces the hypothesis that lower salinity areas of the Tagus estuary might have more ecological niches available for NIS colonization. Since sampling surveys conducted during different periods might have influenced the comparison between establishment inside and outside recreational marinas, a Rapid Assessment Survey as defined by Minchin (2007) conducted in all marinas and neighbouring areas during the same period of the year would help disentangling the dispersal ability of NIS.

The identification of areas of major risk of invasion by NIS is a key priority to support the implementation of regulation that aims to improve ecosystems' conservation, such as the Marine Strategy Framework Directive (MSFD). NIS risk assessments are used to identify high-risk species and/or pathways of introduction and establish management measures (Coop et al., 2016). Nevertheless, the risk of invasion by NIS also depends on the environmental susceptibility of different areas to be colonized. The results of this study provide evidence of the high tolerance of NIS to estuarine gradients, showing that successful invaders have a much wider spatial distribution than native communities. Although low salinity areas seem to have a higher niche availability and greater susceptibility for colonization, upstream areas with a strong freshwater influence were less occupied by NIS, indicating that this might be a limiting factor.

The results of the present study also emphasize the importance of monitoring NIS occurrence in recreational marinas, in order to have an early warning on the arrival of new potential invaders and to prevent its dispersal to sensitive ecosystems.

CRediT authorship contribution statement

I. Afonso: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **E. Berecibar:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **N. Castro:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **J.L. Costa:** Conceptualization, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision. **P. Frias:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **F. Henriques:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **P. Moreira:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **P.M. Oliveira:** Software, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **G. Silva:** Validation, Investigation, Resources, Writing - original draft, Writing - review & editing. **P. Chainho:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Annex 1. . List of *taxa* found inside recreational marinas and respective neighbouring areas (OEI – Oeiras; ALC – Alcântara; PDN – Parque das Nações; VFX – Vila Franca de Xira.

	INSIDE				OUTSIDE			
Taxa	OEI	ALC	PDN	VFX	OEI	ALC	PDN	VFX
Annelida	–	–	–	–	–	–	–	–
Eteone flava	x	–	–	–	–	–	–	–
Harmothoe impar	–	x	–	–	–	–	–	–
Lumbrineridae n.i.	–	x	–	–	–	–	–	–
Marphysa sanguinea	–	x	–	–	–	–	–	–
Nereididae n.i.	–	x	–	–	–	x	–	–
Nereis sp.	–	–	–	–	–	x	–	–
Nereis zonata	x	–	–	–	–	–	–	–
Oxydromus flexuosus	x	–	–	–	–	–	–	–
Perinereis cultrifera	x	–	–	–	–	–	–	–
Phyllodocidae n.i.	x	x	–	–	–	–	–	–
Platynereis dumerili	–	x	–	–	–	–	–	–
Sabellaria sp.	–	x	–	–	–	–	–	–
Sabellidae n.i.	–	x	–	–	–	–	–	–
Serpulidae n.i.	x	x	–	–	–	–	–	–
Sphaerosyllis bulbosa	x	–	–	–	–	–	–	–
Spionidae n.i.	–	x	–	–	–	–	–	–
Spirobranchus lamarcki	x	–	x	–	–	–	–	–
Syllis gracilis	x	–	–	–	–	–	–	–
Syllis sp.	x	x	–	–	–	–	–	–
Terebellidae n.i.	–	x	–	–	–	–	–	–
Terebellomorpha n.i.	–	x	–	–	–	–	–	–
Arthropoda	–	–	–	–	–	–	–	–
Amphibalanus amphitrite	x	x	–	–	–	x	x	–
Amphibalanus improvisus	x	x	x	x	–	–	–	–
Amphilocus sp.	–	–	–	–	–	–	x	–
Amphithoe sp.	–	–	–	–	–	x	–	–
Ampithoe riedli	–	–	x	–	–	–	–	–
Apherusa jurinei	–	–	–	x	–	–	–	–
Apohyale prevostii	–	–	x	–	–	–	–	–
Austrominius modestus	x	x	x	–	–	x	x	–

Balanidae n.i.	–	x	–	–	–	–	–	–
Balanus balanus	–	–	–	–	–	x	–	–
Balanus improvisus	–	–	–	–	–	x	x	–
Balanus trigonus	x	x	–	–	–	x	x	–
Caprella scaura	–	–	–	–	x	x	x	–
Carcinus maenas	–	–	x	–	–	x	x	–
Chironomidae n.i.	x	x	x	x	x	–	x	x
Corophium orientale	–	–	–	x	–	–	–	x
Cyathura carinata	x	x	x	–	–	x	–	–
Decapoda n.i.	–	x	–	x	–	–	–	–
Gammaridae n.i.	–	–	–	–	–	x	–	–
Gammarus subtypicus	–	–	–	x	–	–	–	x
Harpinia pectinata	x	–	–	–	x	–	–	–
Heterotanais oerstedti	–	–	–	–	x	x	–	–
Hyale pontica	–	–	–	–	–	x	–	–
Jassa ocia	–	–	–	–	–	x	–	–
Lekanesphaera hookeri	–	–	x	–	x	–	–	–
Lekanesphaera rugicauda	–	–	–	–	–	–	–	x
Leptocheirus sp.	x	–	–	–	x	x	x	–
Leucothoidae n.i.	–	–	x	–	–	–	–	–
Ligia oceanica	x	x	x	–	–	–	–	–
Lumbrineridae n.i.	x	–	–	–	–	–	–	–
Melita palmata	–	–	–	–	–	x	x	–
Melitidae n.i.	–	–	–	–	–	x	x	–
Microdeutopus armatus	–	–	–	–	–	x	–	–
Monocorophium acherusicum	x	x	x	–	–	x	x	–
Orchestia gammarellus	–	–	x	–	–	–	–	–
Palaemon elegans	x	x	x	–	–	–	–	–
Palaemon longirostris	x	–	x	–	–	–	–	–
Palaemon macrodactylus	–	–	x	–	–	–	–	–
Palaemon serratus	–	–	x	–	–	–	–	–
Perforatus perforatus	–	–	–	–	x	x	x	–
Pilumnus hirtellus	x	–	–	–	–	–	–	–
Pilumnus sp.	–	x	–	–	–	x	–	–
Pinnotheres pisum	x	x	–	–	–	x	x	–
Porcellana platycheles	–	–	–	–		–	x	–
Rhithropanopeus harrisii	x	–	–	–		–	–	–
Tanais dulongii	x	x	–	–		–	x	–
Bryozoa	–	–	–	–		–	–	–
Botryllus sp.	x	–	–	–		–	–	–
Bryozoa sp. 1	x	–	–	–		–	–	–
Bryozoa sp. 2	x	–	–	–		–	–	–
Bugula neritina	x	x	x	–		–	–	x
Conopeum reticulum	–	x	–	–		–	–	–
Conopeum seurati	–	–	x	–		–	–	–
Cryptosula pallasiana	–	x	x	–		–	–	–
Nolella sp.	–	–	10	–		–	–	–
Tricellaria inopinata	x	x	–	–		x	x	x
Watersipora subtorquata	x	x	x	–		x	x	x
Chordata	–	–	–	–		–	–	–
Actinopterygii n.i.	x	–	–	–		–	–	–
Ascidiella aspersa	–	x	–	–		–	–	–
Corella eumyota	–	x	–	–		–	–	–
Microcosmus squamiger	x	x	–	–		x	x	x
Molgula manhattensis	–	x	x	–		–	–	–
Styela clava	–	x	–	–		–	–	–
Styela plicata	x	x	–	–		–	–	–
Tunicata sp. 1	–	–	x	–		–	–	–
Tunicata sp. 2	x	–	–	–		–	–	–
Tunicata sp. 3	–	x	–	–		–	–	–
Tunicata sp. 4	–	x	–	–		–	–	–
Cnidaria	–	–	–	–		–	–	–
Actiniaria n.i.	x	–	x	–		–	x	–
Echinodermata	–	–	–	–		–	–	–
Holothuria sp.	–	x	–	–		–	–	–
Ophiura ophiura	–	–	–	–		x	x	–
Ophiuridae	x	–	–	–		–	x	–
Mollusca	–	–	–	–		–	–	–
Acanthochitona crinita	x	x	–	–		–	–	–
Aplysia sp.	–	x	–	–		–	–	–
Bittium reticulatum	–	–	–	–		x	–	–
Bivalvia sp.	x	–	–	–		–	–	–
Chaetopleura angulata	–	x	–	–		–	–	x
Doris verrucosa	–	x	–	–		–	–	–
Gibbula umbilicalis	–	–	–	–		x	–	–
Hyatella arctica	–	–	–	–		–	x	–
Magallana gigas	x	x	x	–		–	–	–
Mytilus edulis	–	–	–	–		x	–	–

Mytilus galloprovincialis	x	x	x	–	–	x	x	–
Nodipecten sp.	x	x	–	–	–	–	–	–
Ostrea edulis	–	–	–	–	–	x	x	–
Patella depressa	x	x	–	–	x	–	–	–
Patella vulgata	x	–	–	–	–	–	–	–
Pholadidae	x	–	–	–	–	–	–	–
Siphonaria sp.	x	–	–	–	–	–	–	–
Tritia pygmaea	–	–	–	–	x	x	–	–
Nemertea	–	–	–	–	–	–	–	–
Nemertea n.i.	–	x	–	–	–	–	–	–
Platyhelminthes	–	–	–	–	–	–	–	–
Platyhelminthes n.i.	–	x	–	–	–	–	–	–
Porifera	–	–	–	–	–	–	–	–
Porifera n.i.	–	–	x	–	–	x	x	–
Porifera sp. 1	x	x	–	–	–	–	–	–
Porifera sp. 2	x	x	–	–	–	–	–	–
Sipuncula	–	–	–	–	–	–	–	–
Golfingia muricaudata	–	x	–	–	–	x	–	–

References

Amat, J.N., Tempera, F., 2009. *Zoobotryon verticillatum* Della Chiaje, 1822 (Bryozoa), a new occurrence in the archipelago of the Azores (North-Eastern Atlantic). Mar. Pollut. Bull. 58, 761–764. https://doi.org/10.1016/j.marpolbul.2009.02.019.

Aquiloni, L., Ilhéu, M., Gherardi, F., 2005. Habitat use and dispersal of the invasive crayfish Procambarus clarkii in ephemeral water bodies of Portugal. Mar. Freshwater Behav. Physiol. 38 (4), 225–236. https://doi.org/10.1080/10236240500310195.

Ashton, G., Boos, K., Shucksmith, R., Cook, E., 2006. Rapid Assessment of the distribution of marine non-native species in marinas in Scotland. Aquat. Invasions 1, 209–213. https://doi.org/10.3391/ai.2006.1.4.3.

Bacchiocchi, F., Airoldi, L., 2003. Distribution and dynamics of epibiota on hard structures for coastal protection. Estuar. Coast. Shelf Sci. 56, 1157–1166. https://doi.org/10.1016/S0272-7714(02)00322-0.

Bax, N., Hayes, K., Marshall, A., Parry, D., Thresher, R., 2002. Man-made marinas as sheltered islands for alien marine organisms: establishment and eradication of an alien invasive marine species. In: Veitch, C.R., Clout, M.N. (Eds.), Turning the Tide: The Eradication Of Invasive Species. IUCN SSC Invasive Species Specialist Group, IUCN, Gland, Switzerland and Cambridge, UK, pp. 26–39.

Bax, N., Williamson, A., Aguero, M., Gonzalez, E., Geeves, W., 2003. Marine invasive alien species: a threat to global biodiversity. Mar. Policy 27, 313–323. https://doi.org/10.1016/S0308-597X(03)00041-1.

Borsje, B.W., van Wesenbeeck, B.K., Dekker, F., Paalvast, P., Bouma, T.J., van Katwijk, M.M., de Vries, M.B., 2011. How ecological engineering can serve in coastal protection. Ecol. Eng. 37, 113–122. https://doi.org/10.1016/j.ecoleng.2010.11.027.

Bulleri, F., Airoldi, L., 2005. Artificial marine structures facilitate the spread of a non-indigenous green alga, *Codium fragile* ssp. *tomentosoides*, in the north Adriatic Sea. J. Appl. Ecol. 42, 1063–1072. https://doi.org/10.1111/j.1365-2664.2005.01096.x.

Bulleri, F., Chapman, M.G., 2004. Intertidal assemblages on artificial and natural habitats in marinas on the north-west coast of Italy. Mar. Biol. 145, 381–391. https://doi.org/10.1007/s00227-004-1316-8.

Cabral, H., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. Environ. Biol. Fishes 56, 389–397. https://doi.org/10.1023/A:1007571523120.

Canning-Clode, J., Souto, J., McCann, L., 2013b. First record of *Celleporaria brunnea* (Bryozoa: Lepraliellidae) in Portugal and in the East Atlantic. Mar. Biodivers. Rec. 6, e108. https://doi.org/10.1017/S1755267213000821.

Canning-Clode, J., Fofonoff, P., McCann, L., Carlton, J.T., Ruiz, G., 2013a. Marine invasions on a subtropical island: fouling studies and new records in a recent marina on Madeira Island (Eastern Atlantic Ocean). Aquat. Invasions 8, 261–270. https://doi.org/10.3391/ai.2013.8.3.02.

Carlton, J.T., Hodder, J., 1995. Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th Century sailing vessel. Mar. Biol. 121, 721–730. https://doi.org/10.1007/BF00349308.

Chainho, P., A. Botelho, A.C. Costa, A. Teodósio, F. Arenas, J. Canning-Clode, J. Micael, M. Parente, L. Pereira, M.I. Trigo, M.T. Guerra & M. Sousa. 2018. Portuguese national report. In Interim Report of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO), 7–9 March 2018, Madeira, Portugal. ICES CM 2018/HAPISG:11. 179 pp.

Chainho, P., Costa, J.L., Chaves, M.L., Lane, M.F., Dauer, D.M., Costa, M.J., 2006. Seasonal and spatial patterns of distribution of subtidal benthic invertebrate communities in the Mondego River, Portugal – a poikilohaline estuary. Hydrobiologia 555, 59–74. https://doi.org/10.1007/1-4020-4697-9_6.

Chainho, P.A. Amorim, A. Chícharo, F. Arenas, J. Canning-Clode, J.L. Costa, A. Fernandes, J. Micael, A.C. Costa, M. Sousa, R. Sousa, R. Melo, M. Parente, F. Ribeiro & M. Guerra. 2014. Portuguese national report. In Report of the ICES Working Group on Introduction and Transfers of Marine Organisms (WGITMO), 19 - 21 March 2014, Klaipeda, Lithuania. ICES CM 2014/ACOM: 32. 259 pp.

Chainho, P., Fernandes, A., Amorim, A., Avila, S.P., Canning-Clode, J., Castro, J.J., Costa, A.C., Costa, J.L., Cruz, T., Gollasch, S., Grazziotin-Soares, S., Melo, R., Micael, J., Parente, M.I., Semedo, J., Silva, T., Sobral, D., Sousa, M., Torres, P., Veloso, V., Costa, M.J., 2015. Non-indigenous species in Portuguese coastal areas, coastal lagoons, estuaries and islands. Estuar. Coast. Shelf Sci. 167, 199–211. https://doi.org/10.1016/j.ecss.2015.06.019.

Chapman, M., Underwood, A., 2011. Evaluation of ecological engineering of "armoured" shorelines to improve their value as habitat. J. Exp. Mar. Biol. Ecol. 400, 302–313.

Chícharo, M.A., Leitão, T., Range, P., Gutierrez, C., Morales, J., Morais, P., Chícharo, L., 2009. Alien species in the Guadiana estuary (SE-Portugal/SW-Spain): *Blackfordia virginica* (Cnidaria, Hydrozoa) and *Palaemon macrodactylus* (Crustacea, Decapoda): potential impacts and mitigation measures. Aquat. Invasions 4, 501–506. https://doi.org/10.3391/ai.2009.4.3.11.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, U.K.

Cole, E., Keller, R.P., Garbach, K., 2019. Risk of invasive species spread by recreational boaters remains high despite widespread adoption of conservation behaviors. J. Environ. Manage. 229, 112–119.

Costa, M.J., 1999. O estuário do Tejo. Livros Cotovia, Lisboa, Portugal.

Dafforn, K.A., Johnston, E.L., Glasby, T.M., 2009. Shallow moving structures promote marine invader dominance. Biofouling 25, 277–287. https://doi.org/10.1080/08927010802710618.

Davidson, I., Zabin, C., Chang, A., Brown, C.W., Sytsma, M., Ruiz, G., 2010. Recreational boats as potential vectors of marine organisms at an invasion hotspot. Aquat. Biol. 11, 179–191. https://doi.org/10.3354/ab00302.

Derungs, J., 2016. Guia técnico do curso de identificação das espécies de briozoários não indígenas da costa portuguesa. Paço de Arcos, Portugal.

Dyrynda, P., Fairall, V., Occhipinti Ambrogi, A., d'Hondt, J., 2000. The distribution, origins and taxonomy of *Tricellaria inopinata* d'Hondt and Occhipinti Ambrogi, 1985, an invasive bryozoan new to the Atlantic. J. Nat. Hist. 34, 1993–2006. https://doi.org/10.1080/00222930050144828.

Essink, K., Dekker, R., 2002. General patterns in invasion ecology tested in the Dutch Wadden Sea: the case of a brackish-marine polychaetous worm. Biol. Invasions 4, 359–368. https://doi.org/10.1023/A:1023692825663.

Farrapeira, C.M.R., Melo, A., Barbosa, D.F., Silva, K.M.E., 2007. Ship hull fouling in the Port of Recife, Pernambuco. Braz. J. Oceanogr. 55, 207–221. https://doi.org/10.1590/S1679-87592007000300005.

Ferrario, J., Caronni, S., Occhipinti-Ambrogi, A., Marchini, A., 2017. Role of commercial harbours and recreational marinas in the spread of non-indigenous fouling species. Biofouling 33 (8), 651–660.

Floerl, O., Inglis, G.J., 2005. Starting the invasion pathway: the interaction between source populations and human transport vectors. Biol. Invasions 7, 589–606. https://doi.org/10.1007/s10530-004-0952-8.

Foster, V., Giesler, R.J., Wilson, A.M.W., Nall, C.R., Cook, E.J., 2016. Identifying the physical features of marina infrastructure associated with the presence of non-native species in the UK. Mar. Biol. 163 (8), 173.

Galil, B.S., Marchini, A., Occhipinti-Ambrogi, A., Minchin, D., Narščius, A., Ojaveer, H., Olenin, S., 2014. International arrivals: Widespread bioinvasions in European Seas. Ethol. Ecol. Evol. 26, 152–171. https://doi.org/10.1080/03949370.2014.897651.

Gestoso, I., Ramalhosa, P., Oliveira, P., Canning-Clode, J., 2017. Marine protected communities against biological invasions: a case study from an offshore island. Mar. Pollut. Bull. 119 (1), 72–80.

Glasby, T.M., Connell, S.D., 2001. Orientation and position of substrata have large effects on epibiotic assemblages. Mar. Ecol. Prog. Ser. 214, 127–135. https://doi.org/10.3354/meps214127.

Glasby, T.M., Connell, S.D., Holloway, M.G., Hewitt, C.L., 2007. Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? Mar. Biol. 151, 887–895. https://doi.org/10.1007/s00227-006-0552-5.

Jimenez, H., Ruiz, G.M., 2016. Contribution of non-native species to soft-sediment marine community structure of San Francisco Bay, California. Biol. Invasions 18, 2007–2016. https://doi.org/10.1007/s10530-016-1147-9.

Jimenez, H., Keppel, E., Chang, A.L., Ruiz, G.M., 2018. Invasions in marine communities: contrasting species richness and community composition across habitats and salinity. Estuaries Coasts 41, 484–494. https://doi.org/10.1007/s12237-017-0292-4.

Johnson, L.E., Carlton, J.T., 1996. Post-establishment spread in large-scale invasions:

dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. Ecology 77, 1686–1690. https://doi.org/10.2307/2265774.

Johnson, L.E., Ricciardi, A., Carlton, J.T., 2001. Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boats. Ecol. Appl. 11, 1789–1799. https://doi.org/10.2307/3061096.

Kenworthy, J.M., Rolland, G., Samadi, S., Lejeusne, C., 2018. Local variation within marinas: effects of pollutants and implications for invasive species. Mar. Pollut. Bull. 133, 96–106. https://doi.org/10.1016/j.marpolbul.2018.05.001.

Lejeusne, C., Latchere, O., Petit, N., Rico, C., Green, A.J., 2014. Do invaders always perform better? Comparing the response of native and invasive shrimps to temperature and salinity gradients in south-west Spain. Estuar. Coast. Shelf Sci. 136, 102–111. https://doi.org/10.1016/j.ecss.2013.11.014.

Levin, L., 2006. Recent progress in understanding larval dispersal: new directions and digressions. Integr. Comp. Biol. 46, 282–297. https://doi.org/10.1093/icb/icj024.

Locke, A., Hanson, J.M., Ellis, K.M., Thompson, J., Rochette, R., 2007. Invasion of the southern Gulf of St. Lawrence by the clubbed tunicate (*Styela clava* Herdman): potential mechanisms for invasions of Prince Edward Island estuaries. J. Exp. Mar. Bio. Ecol. 342, 69–77. https://doi.org/10.1016/j.jembe.2006.10.016.

Lord, J.P., Calini, J.M., Whitlatch, R.B., 2015. Influence of seawater temperature and shipping on the spread and establishment of marine fouling species. Mar. Biol. 162, 2481–2492. https://doi.org/10.1007/s00227-015-2737-2.

Martínez-Laiz, G., Ulman, A., Ros, M., Marchini, A., 2019. Is recreational boating a potential vector for non-indigenous peracarid crustaceans in the Mediterranean Sea? A combined biological and social approach. Mar. Pollut. Bull. 140, 403–415.

Minchin, D., 2007. Rapid coastal survey for targeted alien species associated with floating pontoons in Ireland. Aquat. Invasions 2, 63–70. https://doi.org/10.3391/ai.2007.2.1.8.

Minchin, D., Floerl, O., Savini, D., Occhipinti-Ambrogi, A., 2006. Small craft and the spread of exotic species. In: Davenport, J., Davenport, J.L. (eds.), The Ecology of Transportation: Managing Mobility for the Environment. Environmental Pollution 10. Springer, Dordrecht, The Netherlands, pp. 99-118.

Mineur, F., Cook, E.J., Minchin, D., Bohn, K., MacLeod, A., Maggs, C.A., 2012. Changing coasts: marine aliens and artificial structures. Oceanogr. Mar. Biol.: Ann. Rev. 50, 189–233. https://doi.org/10.1201/b12157-5.

Murray, C.C., Pakhomov, E.A., Therriault, T.W., 2011. Recreational boating: a large unregulated vector transporting marine invasive species. Divers. Distrib. 17, 1161–1172. https://doi.org/10.1111/j.1472-4642.2011.00798.x.

Occhipinti-Ambrogi, A., Savini, D., 2003. Biological invasions as a component of global change in stressed marine ecosystems. Mar. Pollut. Bull. 46, 542–551. https://doi.org/10.1016/S0025-326X(02)00363-6.

Paavola, M., Olenin, S., Leppäkoski, E., 2005. Are invasive species most successful in habitats of low native species richness across European brackish water seas? Estuar. Coast. Shelf Sci. 64, 738–750. https://doi.org/10.1016/j.ecss.2005.03.021.

Paulay, G., Kirkendale, L., Lambert, G., Meyer, C., 2002. Anthropogenic biotic interchange in a coral reef ecosystem: a case study from Guam. Pacific Sci. 56, 403–422. https://doi.org/10.1353/psc.2002.0036.

Pelletier-Rousseau, M., Bernier, R., Murray, C.C., Drolet, D., Lacoursière-Roussel, A., Locke, A., Simard, N., 2019. Assessment of recreational boating as a vector for marine non-indigenous species on the Atlantic coast of Canada. Biol. Invasions 21 (7), 2447–2470.

Peters, K., Sink, K.J., Robinson, T.B., 2019. Sampling methods and approaches to inform standardized detection of marine alien fouling species on recreational vessels. J. Environ. Manage. 230, 159–167.

Piscart, C., Moreteau, J.C., Beisel, J.N., 2005. Biodiversity and structure of macroinvertebrate communities along a small permanent salinity gradient (Meurthe River, France). Hydrobiologia 551, 227–236. https://doi.org/10.1007/s10750-005-4463-0.

Prato, E., Parlapiano, I., Biandolino, F., 2013. Seasonal fluctuations of some biological traits of the invader Caprella scaura (Crustacea: Amphipoda: Caprellidae) in the Mar Piccolo of Taranto (Ionian Sea, southern Italy). Sci. Mar. 77, 169–178. https://doi.org/10.3989/scimar.03631.21B.

Projecto-Garcia, J., Cabral, H., Schubart, C.D., 2010. High regional differentiation in a North American crab species throughout its native range and invaded European waters: a phylogeographic analysis. Biol. Invasions 12, 253–263. https://doi.org/10.1007/s10530-009-9447-y.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Ramalhosa, P., Camacho-Cruz, K., Bastida-Zavala, R and Canning-Clode, J., 2014. First record of Branchiomma bairdi McIntosh, 1885 (Annelida: Sabellidae) from Madeira Island, Portugal (northeastern Atlantic Ocean). BioInvasions Records 3(3) press.

Remane, A., 1934. Die Brackwasserfauna. Zool. Anz. 7, 34–74.

Remane, A., Schlieper, C., 1971. The Biology of Brackish Waters. Wiley Interscience, New York.

Ros, M., Vázquez-Luis, M., Guerra-Garcia, J.M., 2013. The role of marinas and recreational boating in the occurrence and distribution of exotic caprellids (Crustacea: Amphipoda) in the Western Mediterranean: Mallorca Island as a case study. J. Sea Res. 83, 94–103. https://doi.org/10.1016/j.seares.2013.04.004.

Ros, M., Guerra-Garcia, J.M., Navarro-Barranco, C., Cabezas, M.P., Vásquez-Luis, M., 2014. The spreading of the non-native caprellid (Crustacea: Amphipoda) *Caprella scaura* Templeton, 1836 into southern Europe and northern Africa: a complicated taxonomic history. Mediterr. Mar. Sci. 15, 145–155. https://doi.org/10.12681/mms.469.

Ruiz, G., Fofonoff, P., Carlton, J., Wonham, M., Hines, A., 2000. Invasion of Coastal Marine Communities in North America: apparent patterns, processes, and biases. Annu. Rev. Ecol. Syst. 25, 481–531. https://doi.org/10.1146/annurev.ecolsys.31.1.481.

Ryland, J., Bishop, J., De Blauwe, H., Nagar, A., Minchin, D., Wood, C., Yunnie, A., 2011. Alien species of Bugula (Bryozoa) along the Atlantic coasts of Europe. Aquatic Invasions 6, 17. https://doi.org/10.3391/ai.2011.6.1.03.

Sanders, H.L., Mangelsdorf, P.C., Hampson, G.R., 1965. Salinity and faunal distribution in the Pocasset River, Massachusetts. Limnol. Oceanogr. 10, R216–R229. https://doi.org/10.4319/lo.1965.10.suppl2.r216.

Seebens, H., Gastner, M.T., Blasius, B., 2013. The risk of marine bioinvasion caused by global shipping. Ecol. Lett. 16 (6), 782–790.

Shucksmith, R.J., Shelmerdine, R.L., 2015. A risk based approach to non-native species management and biosecurity planning. Mar. Policy 59, 32–43. https://doi.org/10.1016/j.marpol.2015.05.001.

Simkanin, C., Dower, J.F., Filip, N., Jamieson, G., Therriault, T.W., 2013. Biotic resistance to the infiltration of natural benthic habitats: examining the role of predation in the distribution of the invasive ascidian *Botrylloides violaceus*. J. Exp. Mar. Biol. Ecol. 439, 76–83. https://doi.org/10.1016/j.jembe.2012.10.004.

Simkanin, C., Davidson, I., Therriault, T., Jamieson, G., Dower, J., 2017. Manipulating propagule pressure to test the invasibility of subtidal marine habitats. Biol. Invasions 19, 1565. https://doi.org/10.1007/s10530-017-1379-3.

Sousa, R., Rufino, M., Gaspar, M., Antunes, C., Guilhermino, L., 2008. Abiotic impacts on spatial and temporal distribution of Corbicula fluminea (Müller, 1774) in the River Minho estuary, Portugal. Aquatic Conserv: Mar. Freshw. Ecosyst. 18, 98–110. https://doi.org/10.1002/aqc.838.

Sousa, R., Gutierrez, J.L., Aldridge, D.C., 2009. Non-indigenous invasive bivalves as ecosystem engineers. Biol. Invasions 11, 2367–2385. https://doi.org/10.1007/s10530-009-9422-7.

Souto, J., Ramalhosa, P., Canning-Clode, J., 2018. Mar. Biodiv. 48, 977. https://doi.org/10.1007/s12526-016-0592-0.

Tyrrell, M.C., Byers, J.E., 2007. Do artificial substrates favor nonindigenous fouling species over native species? J. Exp. Mar. Bio. Ecol. 342, 54–60. https://doi.org/10.1016/j.jembe.2006.10.014.

Ulman, A., Ferrario, J., Forcada, A., Seebens, H., Arvanitidis, C., Occhipinti-Ambrogi, A., Marchini, A., 2019. Alien species spreading via biofouling on recreational vessels in the Mediterranean Sea. J. Appl. Ecol.

Vaz-Pinto, F., Torrontegi, O., Prestes, A.C.L., Alvaro, N.V., Neto, A.I., Martins, G.M., 2014. Invasion success and development of benthic assemblages: effect of timing, duration of submersion and substrate type. Mar. Environ. Res. 94, 72–79. https://doi.org/10.1016/j.marenvres.2013.12.007.

Wasson, K., Zabin, C.J., Bedinger, L., Diaz, M.C., Pearse, J.S., 2001. Biological invasions of estuaries without international shipping: the importance of intraregional transport. Biol. Conserv. 102 (2), 143–153. https://doi.org/10.1016/S0006-3207(01)00098-2.

Webb, J.A., Keough, M.J., 2000. Effects of two marinas on the composition of fouling assemblages. Biofouling 16, 345–360. https://doi.org/10.1080/08927010009378457.

Ysebaert, T., Herman, P.M.J., Meire, P., Craeymeersch, J., Verbeek, H., Heip, C.H.R., 2003. Large-scale spatial patterns in estuaries: Estuarine macrobenthic communities in the Schelde estuary, NW Europe. Estuar. Coast. Shelf Sci. 57, 335–355. https://doi.org/10.1016/S0272-7714(02)00359-1.

Zabin, C.J., Ashton, G.V., Brown, C.W., Davidson, I.C., Sytsma, M.D., Ruiz, G.M., 2014. Small boats provide connectivity for nonindigenous marine species between a highly invaded international port and nearby coastal harbors. Manage. Biol. Invasion 5, 97–112. https://doi.org/10.3391/mbi.2014.5.2.03.