



UNIVERSITÀ  
DI PAVIA

SCUOLA DI ALTA FORMAZIONE DOTTORALE  
MACRO-AREA SCIENZE E TECNOLOGIE

Dottorato di Ricerca in Scienze della Terra e dell'ambiente

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# **Colonization success of marine non-indigenous species: Monitoring and manipulative approaches**

Anno Accademico 2020-2021  
Ciclo XXXIV

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## Summary

Non-indigenous species (NIS) are considered a global threat to ecosystems with both ecological and economic impacts. Several factors have powerful influences on the bioinvasion process, including physical-chemical constraints and alien-native species interactions. In the marine environment, humans can mediate biological invasions through several vectors like navigation, aquaculture, and artificial canals – e.g. the Suez Canal and ports. Ports are considered hotspot areas of NIS introduction due to higher propagule pressure by ship transport, large abundance of artificial substrates, which provide colonizable habitats, and the capability of NIS to tolerate polluted environments. In the Mediterranean Sea, more than seven hundred multicellular NIS have already been recorded, but this number can be underestimated because monitoring surveys for NIS have not yet been carried out on regular basis. In Italy, although a lot of records on marine NIS have been published, ad-hoc studies on their mechanisms of introduction, possible impacts on local ecosystems and standardized assessment are still lacking, while they are considered essential to correctly implement management measures. This Ph.D. thesis shows the results of a three-year project focused on the assessment of: fouling community composition in areas affected by different vectors of NIS introduction: shipping (by commercial, passenger and recreational vessels) and aquaculture (Aim 1); the “biotic resistance” hypothesis in fouling communities, i.e. the comparative success of NIS colonization in pristine communities (Aim 2); and the control by predators on NIS in fouling assemblages (Aim 3). After a part on explanation of marine NIS (Chapter 1) and one on the detailed description of the Ph.D. project (Chapter 2), the thesis continues with the description of the aim 1 (Chapter 3), aim 2 (Chapter 4) and aim 3 (Chapter 5). Finally, Chapter 6 shows the main findings and conclusion of the Ph.D. project. A three-year monitoring in target sites (aim 1) was done to: i) identify how each port or aquaculture site is interested by NIS colonization, ii) evaluate changes in early stages of fouling community along three years (2018-2020) and iii) detect new NIS at early stages of colonization. The monitoring programme started in July 2018 in the Gulf of La Spezia (Ligurian Sea, Italy) under the supervision of the Smithsonian Environmental Research Center (SERC, USA), following a standard protocol adopted by the Marine Global Earth Observatory (MarineGEO). The experiment was the first one to use the MarineGEO protocol in the Mediterranean Sea. The three-year monitoring survey was conducted with the deployment of a total of 50 PVC panels per year in five different sites (a commercial harbor, three marinas and a site in the proximity of a shellfish farm). A total of 79 taxa were identified, including 11 NIS, ranging from zero to seven NIS for each panel. In comparison with previous surveys, new NIS arrivals were observed in the Gulf of La Spezia: *Botrylloides* cf. *niger*, *Branchiomma* sp., *Branchiomma luctuosum*, *Paraleucilla magna*, and *Watersipora arcuata*. At the end of the monitoring, mean richness and percent cover of NIS were measured, and both measures

significantly differed across the monitoring sites, with higher values in two marinas and in the commercial harbor. Among years, richness of NIS was relatively stable at each monitoring site. The structure of the fouling was influenced more by native and cryptogenic species than by NIS. Moreover, among the monitoring sites, the density of artificial structures was not a reliable predictor or proxy for local NIS abundance. This first application of the SERC method in the Mediterranean Sea, demonstrates both pros and cons, as the detection of new NIS, although a long time for the analysis is needed. Further direct comparisons with other NIS monitoring tools are recommended, and additional tests to assess its effectiveness in this biogeographical area are encouraged. A broader application of this and other standard methods across temporal and spatial scales in the Mediterranean basin should be implemented, providing critical data needed to assess changes in the structure of fouling communities.

The second aim of this project featured the testing of the “biotic resistance” hypothesis - i.e. the capability of healthy and diverse native communities to limit the success of NIS - in fouling communities. Fouling communities occurring in sites lightly or no affected by anthropogenic impacts are composed by a higher number of native species and a lower number of NIS, in contrast with areas more affected by anthropogenic impacts. Nevertheless, evidences of high abundance of NIS in pristine areas have been reported in the literature. The capability of fouling communities growing in a Ligurian Natural Park (Palmaria Island, Gulf of La Spezia) to act as “natural barrier” against NIS introduction and settlement was investigated through a transplant experiment performed in 2019. The same experimental design was repeated in 2020 in the province of Leghorn (North Tyrrhenian Sea, Italy), by selecting as “pristine” site the locality of Chioma, a little marina with very few boats and good environmental conditions. Differences between conditions (transplanted and not-transplanted communities) were statistically significant for the variables “NIS/native species ratio” and “NIS number”. Moreover, the multivariate analyses on the community structure highlighted the importance of some NIS and native species in the differentiation of conditions. Our results indicate a signal of biotic resistance, but further studies should be conducted to clarify species interactions in harbor communities and to identify solutions for reducing NIS colonization in ports.

The control by predators on fouling NIS is a comparatively less known aspect of marine bioinvasions that is worth further investigation. It has recently been investigated in few studies conducted overseas on artificial substrate communities, but no data are available at the moment for the Mediterranean Sea. Therefore, the third aim of this Ph.D. project addressed the effect of predators on the population control of fouling NIS grown on experimental artificial substrates. The caging experiment of predator exclusion was set up in a site (Santa Teresa Bay) inside the Gulf of La Spezia in August 2020 and

lasted 70 days. For this experiment, 21 panels and bricks and associated plastic cages were hang on the only floating pontoon of the bay, in order to provide similar environmental conditions to all the panels. The panels were divided in three experimental conditions (seven per condition): “control panels”, “half-caged panels” and “caged panels”, in order to assess the predation effect on the fouling assemblages growing onto the panels. Predation effect was observed: for two investigated variables (i.e., Shannon-index and cover of native species). Moreover, analysing the whole fouling assemblages through multivariate analyses, predation showed an influence on the development of fouling assemblages. Nevertheless, a more powerful experiment will provide further results, in order to confirm or not our conclusion. This Ph.D. project has studied, by means of standard monitoring methodology, robust and valuable information on invasion biology of some components of the fouling communities of urbanized coastal sites. Furthermore, it has been conducted manipulative ecological experiments, which can shed new light on some mechanisms underlying the process of invasion, that is still underway in the Mediterranean Sea. Many questions are still open and need further efforts to be properly understood, but the results here showed could pave the way for future research. During this Ph.D. period, a paper on the first aim was published in 2021 and a paper on the second aim was submitted in November 2021.

# 1. Marine non-indigenous species

## 1.1 Definition, vectors, pathways and potential impacts

As stated by the International Union for Conservation of Nature, introduced or non-indigenous species (NIS) are “species, subspecies or variety or cultivar or breed, moved intentionally or unintentionally by human activities beyond the limits of its native geographic range, or resulting from breeding or hybridization and being released into an area in which it does not naturally occur” (IUCN 2020). NIS are one of the major threats to native biodiversity, including in the marine realm (Occhipinti-Ambrogi 2007, Britton-Simmons & Abbott 2008, Olenin et al. 2011, Johnston et al. 2017), and have a huge economic impact worldwide (Diagne et al. 2021). Over the past five centuries, thousands of species were moved around the world in several different ways (Carlton 1989).

Assessing the biogeographic status of a marine species - i.e., native or non-indigenous species - can be a hard challenge. Several criteria were therefore introduced (e.g. Chapman & Carlton 1991) and the genetic approach can help the classification of species. Moreover, the concept of “cryptogenic species”, introduced by Carlton in 1982 to help the species status classification, indicates a species “that is not demonstrably native or introduced” (Carlton 1996). Nevertheless, uncertainty in the status assessment is a common result (Katsanevakis & Moustakas 2018), and for this reason the biogeographic classification of species is continuously under revision, with several and continuous reviews on the assessment of biogeographic status.

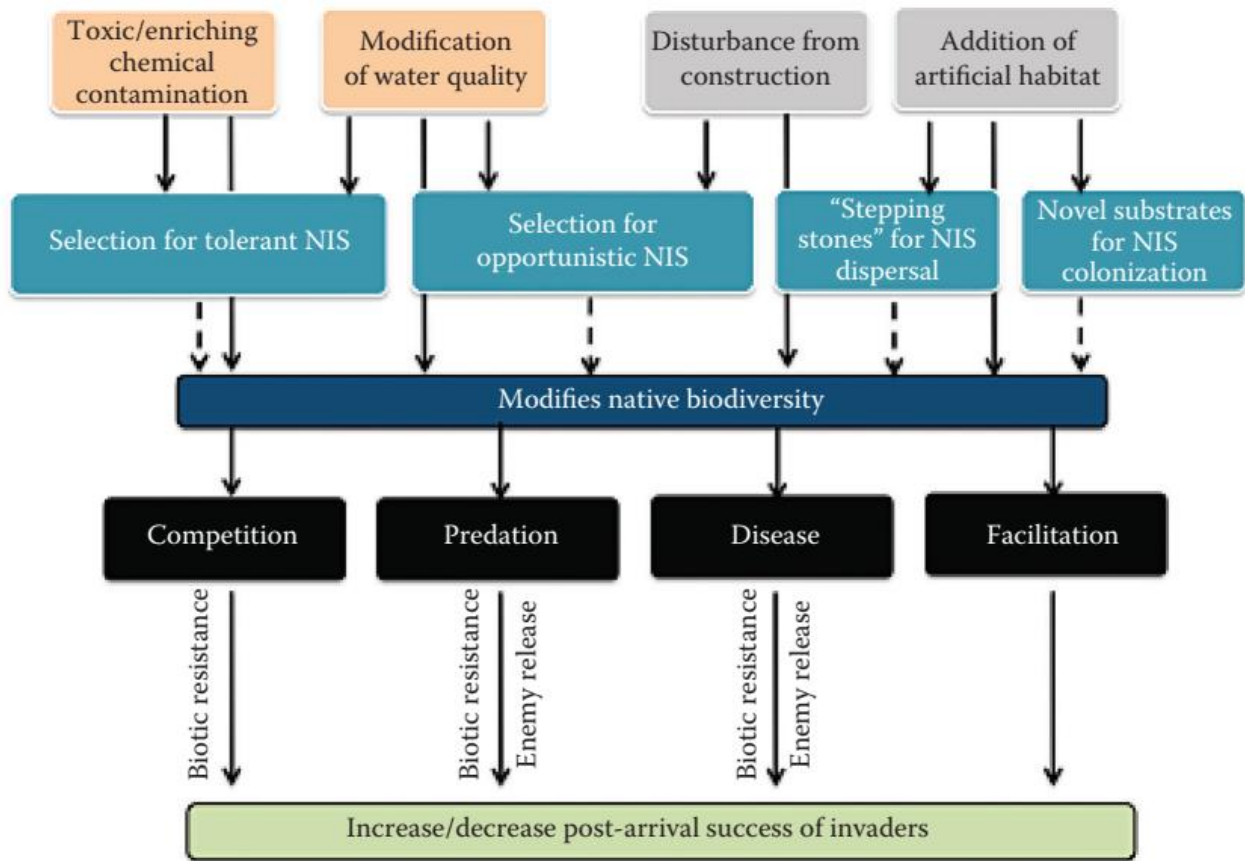
Six main phases of the NIS introduction process can be distinguished (Colautti & MacIsaac 2004, Colautti et al. 2006, Occhipinti-Ambrogi 2007), starting from the concept of propagule pressure, i.e. “the number of invading ‘propagules’ for a given introduction, and the frequency with which they are introduced” (Williamson & Fitter 1996, Colautti & MacIsaac 2004) and continuing with the adaptation to the environmental conditions. Propagules are larvae, seeds, spores, and fragments of colonial specimens that can be transported, causing an introduction event. Each phase (or stage) presents a series of “filters” that may preclude the passage of non-indigenous specimens to subsequent phases (Colautti & MacIsaac 2004):

- Stage 0: a new potential NIS begin as resident in a donor region.
- Stage I: the NIS propagules are collected by a vector.
- Stage II: the NIS propagules are introduced in a novel environment (if they survive to the transport).
- Stage III: NIS become established in the novel environment (if they survive to pre- and post-settlement phases).

- Stage IV
  - a): NIS is widespread in the novel environment.
  - b): NIS is dominant in the novel environment.
- Stage V: NIS is widespread and dominant in the novel environment.

The “filters” acting during each stage can be summarized in three main categories: intensity of the propagule pressure; physical-chemical characteristic of novel environments; and community interactions (Colautti et al. 2006). Focusing on the post-arrival phases, chemical contamination, water quality modification (e.g. salinity or temperature), disturbance from new construction (e.g. the increase of water turbidity), and addition of artificial habitat in the recipient region are the main factors that can positively or negatively affect the establishment of a NIS (Johnston et al. 2017; Figure 1). These factors can also interfere with the ecological interactions involved in the bioinvasion processes, namely competition, predation, and facilitation (Johnston et al. 2017). Although these interactions were largely studied in the past years (e.g., Stachowicz et al. 1999, 2002, Simkanin et al. 2013, Freestone et al. 2013), the ecological mechanisms have to be completely understood, but a pivotal role in determining the success or failure of NIS growth was suggested (Stachowicz et al. 2002). In chapter four and five of this thesis, two of these interactions will be discussed in more detail, namely the discussion on “biotic resistance hypothesis” (Elton, C.S. 1958, Levine & D’Antonio 1999, Bruno et al. 2003, Fridley et al. 2007, Kimbro et al. 2013) and the role of predation in marine communities (Paine 1966, Lubchenco 1986, Lafferty & Suchanek 2016), working on NIS in fouling assemblages (Torchin et al. 2021).





**Figure 1.1** “Chemical (orange) and physical (grey) disturbances from human activities that influence the post-arrival success of invaders. Effects of disturbance on NIS can be direct (dark green) or indirect (blue) and can affect associated biotic processes (black). Direct interactions are illustrated by an unbroken line; indirect interactions are illustrated by a broken line.” (Johnston et al. 2017). License CC BY-NC-ND 4.0.

In the marine realm, there are several NIS vectors and the most important ones are shipping, aquaculture and the construction of artificial canals, which allows the connection of basins biogeographically separated (Ruiz et al. 2000, Occhipinti-Ambrogi & Savini 2003, Katsanevakis et al. 2014, Galil et al. 2014, 2015, 2018b, Tsiamis et al. 2020). The global aquarium trading of species can be considered another vector of NIS diffusion (Guidetti et al. 2015), including aquarium spill-over (Meinesz & Hesse 1991, Meinesz et al. 2001) and live bait trade (Sá et al. 2017).

Shipping is involved in the transport of NIS in two possible ways: propagules can be transported through ballast water (i.e., pumped seawater necessary to stabilize ships at sea) of large vessels or specimens can be transported attached to boat hulls as biofouling, the unwanted accumulation of organisms on submerged structures. For this reason, NIS spread is due to both large commercial and small recreational vessels: the first ones are responsible for the primary introduction in a novel environment (i.e. a commercial harbor), while the second ones for further spreading in the areas nearby (Ruiz et al. 2000, Clarke Murray et al. 2011, Canning-Clode et al. 2013, Zabin 2014, Ferrario et al. 2017, Galil et al. 2018b). Recently, the ballast water charge and discharge has been regulated

by the International Maritime Organization through the Ballast Water Management Convention (IMO 2007), in order to minimize the uptake and release of NIS propagules. On the other hand, the biofouling vector is still mostly unregulated (Clarke Murray et al. 2011, Lehtiniemi et al. 2015), with the exception of Australia, Canada and New Zealand (Lane et al. 2018, Georgiades 2020).

Aquaculture can promote the introduction of NIS both intentionally and accidentally. Selected NIS can be intentionally introduced for aquaculture purposes, e.g., oysters, mussels and salmonids (Minchin 2007, Savini et al. 2010, Zenetos et al. 2012). Furthermore, other NIS associated with cultured species may be also unintentionally (i.e. accidentally) introduced, for example as epibionts (Minchin 2007, Bannister et al. 2019, Tsiamis et al. 2020), or in water tanks and packaging of cultured species and their seedlings shipped for restocking (Savini et al. 2010).

The construction of artificial canals allows the connection between different marine biogeographic regions, and thus it is considered another NIS vector. The Mediterranean Sea is negatively affected by the Suez Canal, a canal opened in 1869 connected to the Red Sea and considered one of the most powerful corridors for marine bioinvasions in the world. In the Mediterranean, almost the half of NIS are introduced through this pathway (Galil et al. 2017, 2018b). In general, the knowledge of pathways and vectors is obviously crucial in understanding the introduction dynamics, but only rarely experimental evidences are available in order to document how a species has been introduced (Zenetos et al. 2012).

A widespread and/or dominant NIS in a novel environment can cause both ecological and economic impacts, becoming invasive. Assessing the magnitude of the impact can be difficult, due to a generalized lack of knowledge (Ojaveer et al. 2015, Occhipinti-Ambrogi 2021, Katsanevakis et al. 2014a). An attempt to quantify NIS impacts was made introducing several indexes for the impact assessment. For example, the Environmental Impact Classification for Alien Taxa (EICAT), the ALien Biotic IndEX (ALEX), and the INvasive Species Effects Assessment Tool (INSEAT) are indexes that summarize the NIS impact in the ecosystems. As expected, each of this indexes shows both advantages and disadvantages in the assessment of real impacts (Occhipinti-Ambrogi 2021).

In general, NIS can cause both ecological and economic impacts. Firstly, NIS have the ability to induce changes in ecosystems physically or chemically, or modifying the ecological interactions, e.g. through competition for resources, grazing/predation, trophic cascading effects, filling up of empty niches and import of parasites and diseases (Wallentinus & Nyberg 2007, Occhipinti-Ambrogi 2021). Secondly, NIS can cause also economic impacts, modifying ecosystem services and goods (Wallentinus & Nyberg 2007) or directly produce economic damages (Galil et al. 2018b). A global

annual cost of biological invasion (including all realms) was estimated in 26.8 billion of US dollars, but this amount is probably underestimated (Diagne et al. 2021). A list of the ecosystem services potentially affected by NIS (Wallentinus & Nyberg 2007) and one on their economic impacts (based on a research conducted on NIS in Chile by Araos et al. 2020) are shown in Tables 1.1-1.2.

Moreover, many marine plants, macroalgae and sessile invertebrates have a profound architectural importance for the ecosystem structure and may play positive impacts, acting as habitat-forming species, nevertheless if they are NIS (Wallentinus & Nyberg 2007). Nevertheless, more studies are needed to assess the ratio between positive and negative impacts of NIS introduction. It must be also considered that NIS removal from a novel environment is a very hard challenge. A precautionary approach on NIS introduction was therefore proposed, which helps both to prevent new NIS introduction and to correctly categorize NIS impacts (Ojaveer et al. 2015).

**Table 1.1 “Examples of ecological services provided by different ecosystems, which can be affected by non-indigenous species” (Wallentinus & Nyberg 2007).**

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O <sub>2</sub> production/absorption
CO <sub>2</sub> absorption/production
N <sub>2</sub> fixation
Storage of nutrients, etc.
Regeneration of nutrients, etc.
Denitrification (shunt for eutrophication)
Possibilities of bioremediation
Trapping of sediment
Protection of shoreline against erosion, flooding, etc.
Filter for land runoff
Clear water by filtering capacity
Shelter for many organisms incl. temporary commercial species
Nursery ground for juveniles from other systems (mobile links)
Provide organic material, nutrients and food to other systems
Records of pollutants (can be used in monitoring)
Scientific and educational information
Recreational values
Aesthetic and artistic values

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**Table 1.2 Example of overall economic impacts caused by seven alien species in Chile (from Araos et al. 2020).**

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Public resources allocated to research
Impact on livestock production
Impact on wood production
Impact on forest plantations
Impacts on components of biodiversity
Resources used in species control
Impact on agricultural production
Loss of forest biomass
Repair of road infrastructure
Potential cost in fire control
Impact on viticulture production
Decrease in carbon sequestration

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## **1.2 NIS in the Mediterranean Sea**

The Mediterranean Sea (meaning “in the middle of the land”) is the largest (2,969,000 km<sup>2</sup>) and deepest (average 1,460 m, maximum 5,267 m) enclosed sea on Earth, located at the crossroads of Africa, Europe, and Asia, where many civilizations flourished and declined in the past millennia (Coll et al. 2010). It is connected with the Atlantic Ocean through the Strait of Gibraltar; with the Sea of Marmara and the Black Sea through the Bosphorus Strait; and with the Red Sea and the Indian Ocean through the artificial Suez Canal. Furthermore, the Strait of Sicily divides the sea into two major basins, the Western and Eastern Mediterranean (Galil et al. 2018b a).

The Mediterranean Sea can be considered a biodiversity hot spot, contributing with 7% of the world marine species diversity (about 17,000 described marine species) in a relatively small biogeographic region (Coll et al. 2010, Galil et al. 2018b). Moreover, the Mediterranean coastlines show high density of inhabitants from 21 modern states, and it is one of the most visited destinations in the world, with about 200 million tourists per year (Coll et al. 2010). For this reason, nowadays habitat loss and degradation, followed by fishing impacts, pollution, climate change, eutrophication, and the establishment of NIS are the main drivers of change in the Mediterranean peculiar biodiversity (Coll et al. 2010).

In particular, biological invasions by NIS in the Mediterranean have started to be studied since the opening of the Suez Canal, but broader studies have begun only from the late 20<sup>th</sup> century, when the huge impacts of the “killer alga” *Caulerpa taxifolia* (M.Vahl) C.Agardh, 1817 were noted along the Western Mediterranean coastal areas (Meinesz & Hesse 1991, Galil et al. 2018b). In the last years, almost 800 multicellular NIS were recorded in the Mediterranean (Rilov & Galil 2009, Galil et al. 2018b), leading this Sea to be one of the most invaded marine provinces in the world (Bailey et al.

2020). Records on NIS presence were compiled as national reports in several countries facing the Mediterranean, like France, Italy and Greece (Olenin et al. 2010, Galil et al. 2018b, ICES 2019). Moreover, a lot of initiatives are taken to oppose NIS introduction in the Mediterranean Sea, like the CIESM Atlas of Exotic Species in the Mediterranean (<https://www.ciesm.org/online/atlas/index.htm>; accessed January 2022), the RAC/SPA program (<https://www.rac-spa.org/aliens>; accessed January 2022) and ICES guidelines (ICES 2005). Nevertheless, a lot has to be done to really mitigate NIS introduction (Lehtiniemi et al. 2015).

NIS introduction in the Mediterranean Sea is mainly due to the Suez Canal (63% of NIS), followed by shipping and aquaculture (Galil et al. 2018b). More in detail, primary introduction through the Suez Canal has affected first of all the Eastern Mediterranean (i.e. the Levantine Basin), while primary introduction through aquaculture related processes has affected some areas more than others. Primary introduction by shipping has interested mainly international and larger ports, while secondary spreading has been favoured by smaller vessels. Moreover, global climate changes is recently promoting areal expansion of NIS confined in the Levantine Basin for many years (Galil et al. 2018a).

In Italy, the last report on NIS presence (GSA-SIBM 2021) indicated a total number of 243 marine and brackish NIS recorded along the 7,375 km of the Italian coasts, belonging to 13 (sub-)Phyla. In Table 1.3 a comparison among NIS presence in the last national report is shown. In 2016, recorded NIS were 186 (GSA-SIBM 2016), while 210 in 2018 (GSA-SIBM 2018), indicating an increasing trend in the last years. These reports can be subject to change, due to the continuing revision of biogeographical status of the species, thanks to the advance of knowledge in the study of the original geographic ranges of the species.

**Table 1.3 Number of NIS recorded along Italian coasts in recent years (2016, 2018 and 2021), grouped by taxonomic macro groups.**

<b>Group</b>	<b>Recorded NIS</b>		
	<b>2016</b>	<b>2018</b>	<b>2021</b>
Macrophyta	55	58	63
Foraminifera	-	-	1
Porifera	1	1	1
Ctenophora	1	2	2
Cnidaria	10	13	14
Nematoda	1	1	1
Platyhelminthes	1	1	1
Polychaeta	18	15	25
Mollusca	33	34	38
Arthropoda	36	43	46
Bryozoa	10	12	12
Tunicata	7	9	13
Vertebrata	13	21	26
<i>Total</i>	<i>186</i>	<i>210</i>	<i>243</i>

### **1.3 Ports as hot-spot areas for NIS: the study of fouling communities**

As stated in paragraph 1.1, shipping can be considered one of the most important vectors for NIS introduction all over the world, considering both ballast water and vessel hulls as possible pathways. In many cases, the first destination of a new-introduced NIS is therefore a port: in commercial harbours the large vessels can transport NIS from distant donor regions, while the smaller touristic marinas are mostly involved in the secondary spread of NIS, thanks to the movements of recreational boats on regional scale (Minchin et al. 2006, Occhipinti-Ambrogi 2007, Floerl et al. 2009, Ferrario et al. 2017, Zettler 2021, Outinen et al. 2021). Recently, recreational vessels were also recognized as important as large vessels in the primary introduction of NIS (Ferrario et al. 2017, Ulman et al. 2019a).

Port habitats are constituted by different artificial structures (e.g. concrete docks, floating pontoons, buoys), which are suitable surfaces for the colonization by hard-bottom species (Wahl 2009), that form the so-called biofouling (IMO 2012). In the macrozoobenthic component of fouling communities both sessile species (e.g. sponges, serpulids, bivalves, barnacles, bryozoans, tunicates), and mobile species (like but not limited to flatworms, amphipods, isopods, sea spiders and brittle stars) are commonly found (Connell & Glasby 1999, Glasby & Connell 1999, Connell 2001b, Lord et al. 2015, Gavira-O'Neill et al. 2018, Culver et al. 2021).

In the colonization process of artificial structures, it has been noted that NIS are often more successful than native species (Glasby et al. 2007, Dafforn et al. 2009, Airoidi et al. 2015). In general NIS are

more common in sheltered environments such as harbours and marinas than in open coast localities (Rius 2011).

Carefully assessing macrofouling assemblages can be very challenging: fouling communities are patchy and show high variability in terms of species diversity and abundance even at small scale (Canning-Clode et al. 2014). For this reason, a suitable sampling plan is fundamental to collect as best as possible samples that represent the whole community, balancing number of sampling replicates and available resources (i.e. time, budget, team size, ...) to conduct the study (Canning-Clode et al. 2014).

In the last decades fouling assemblages have served as a model not only for the study of several ecological issues, like ecological succession (Relini et al. 1994, Perkol-Finkel et al. 2008, Canning-Clode et al. 2009, Lezzi & Giangrande 2018, Pica et al. 2019), but also for the study of bioinvasion processes (Ruiz et al. 2009; Canning-Clode et al. 2011; Marraffini et al. 2017; Marasinghe et al. 2018; Vieira et al. 2018; Leclerc et al. 2018; Ferrario et al. 2020).

Many different methodologies for sampling fouling communities in port habitats have been developed from the second half of the last century, and they will be discussed in detail in paragraph 3.1.1.

## 2. PhD project

### 2.1 Aims of the project

This Ph.D. project is focused on the assessment of: fouling community composition in areas affected by different vectors of NIS introduction, like shipping (by commercial, passenger and recreational vessels) and aquaculture (aim 1); the “biotic resistance” hypothesis in fouling communities where native assemblages may limit the development of NIS populations (aim 2); and the control by native predators on fouling NIS (aim 3).

A yearly monitoring in target sites (aim 1) was settled to: i) identify how each site is interested by NIS colonization, ii) evaluate changes in early stages of fouling community in three years (2018-2020) and iii) early detect new NIS. The monitoring program started in July 2018 in the Gulf of La Spezia (Ligurian Sea, Italy) under the supervision of the Smithsonian Environmental Research Center (SERC, USA), following a standard protocol (Chang et al. 2018) adopted by the Marine Global Earth Observatory (MarineGEO, <https://marinegeo.si.edu/>; accessed October 22<sup>th</sup>, 2021), a Smithsonian-led international coastal research network. The experiment settled in the Gulf of La Spezia was the first one to use the MarineGEO protocol in the Mediterranean Sea. The monitoring was repeated in 2019 and in 2020.

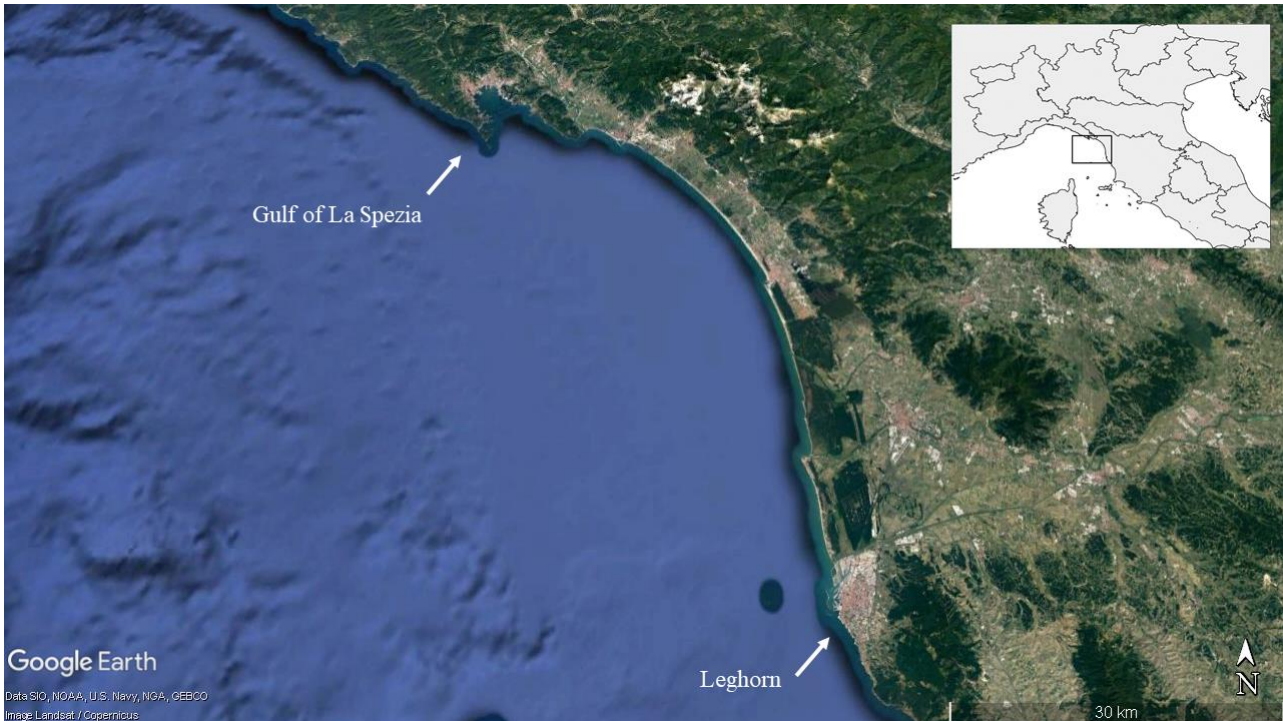
As second aim of this project, the “biotic resistance” hypothesis - i.e. the capability of healthy and diverse native communities to limit the success of NIS (Elton, C.S. 1958, Levine & D’Antonio 1999, Gestoso et al. 2017) - was tested in fouling communities. Fouling communities occurring in sites lightly or not affected by anthropogenic impacts (herein defined as “pristine” sites) are presumably composed by a higher number of native species and a lower number of NIS, in contrast with areas more affected by anthropogenic impacts. The capability of fouling communities growing in a Ligurian Natural Park to act as “natural barrier” against NIS introduction and settlement was investigated through a transplant experiment in marinas in 2019. The same experimental design was repeated in 2020 in the province of Leghorn (North Tyrrhenian Sea, Italy), choosing a nearby pristine area as donor site.

The third aim of the project addressed the effect of predators on the population control of fouling NIS grown on experimental artificial substrates. The control by native predators, including grazing, on fouling NIS is indeed another aspect of marine bioinvasions never investigated in the Mediterranean Sea, while several studies have been conducted overseas (Brown & Swearingen 1998, Anderson & Connell 1999, Connell 2001a, Kremer & da Rocha 2016, Leclerc & Viard 2018). The experiment of this aim was carried out in a single site inside the Gulf of La Spezia in the late summer of 2020.



## 2.2 Study areas

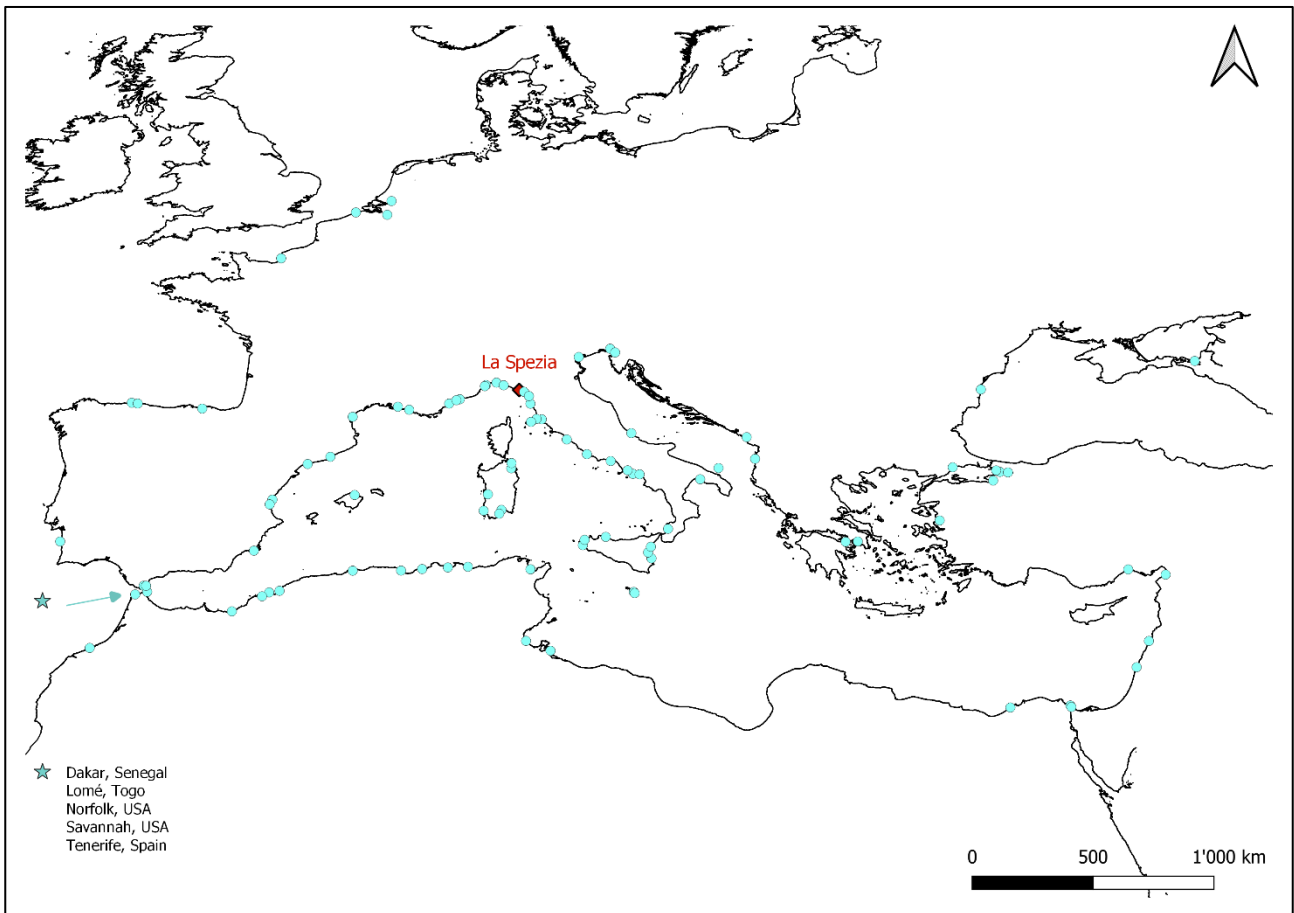
All the experiments of this project were conducted in the Gulf of La Spezia, and only one was replicated also in the Leghorn area (Figure 2.1).



**Figure 2.1** Satellite image of the two study areas investigated in this thesis, modified from Google Earth Pro (v. 7.3.4.8248).

### 2.2.1 The Gulf of la Spezia

Experimental activities related to aim 1 and 3 were conducted in this study area (Figure 2.1), while aim 2 activities were conducted here and along Leghorn coast. Several anthropogenic activities and impacts characterize the Gulf area, including several marinas, naval industries, a military base, an electric power-plant, aquaculture facilities and a commercial harbor, that hosts commercial vessels from all the Mediterranean Sea and the Atlantic Ocean (Fig. 2.2).



**Figure 2.2** Ports of origin (light blue circles) of the ships passing through the port of La Spezia (red diamond) in 2020. Star indicates ports not shown in the map (dataset kindly provided by *Autorità Di Sistema Portuale Mar Ligure Orientale*).

### 2.2.2 Leghorn area

The experiment within aim 2 was also conducted in this study area. Leghorn is one of the main ports in the Tyrrhenian Sea with more than 16 million tons of materials passed through the harbor in the first half of 2017 (<https://www.portaltotirreno.it/wp-content/uploads/2017/09/Porto-di-Livorno-Dati-statistici-I-sem-2017.pdf>; accessed January 2022), that can be considered a hotspot for NIS introduction (Tempesti 2020).

Nearby the city, there are several inlets and small marinas dedicated to locals, with putative low NIS pressure, where iconic species like *Corallium rubrum* (Linnaeus, 1758) are present (Santangelo et al. 2012).

### **2.3 General methodology: SERC protocol**

The monitoring protocol developed by the Smithsonian Environmental Research Center (SERC, USA) has been developed to assess the occurrence, distribution, and diversity of NIS across time and space. The first application started in Chesapeake Bay in 1994 and since then, over 130 separate surveys were conducted in several bays in the US, Canada, Australia, Belize, Panama, Ecuador, and more (<https://serc.si.edu/research/projects/large-scale-surveys-fouling-zooplankton-and-soft-sediment-benthic-habitats>; accessed July 2021). Thanks to these surveys, it was possible to study the diversity of fouling communities, how they change over time, and how new species can affect the community structure, composition and abundance (Marraffini et al. 2017, Tracy et al. 2017, Chang et al. 2018, Carlton 2019).

The method consists in the hanging of PVC panels (14 x 14 cm) from docks, pontoons, or any artificial structures, allowing the settlement and growth of species in an immersion period of three-month, in spring/summer. Panels can therefore act as standardized passive collectors and can provide several information on fouling communities, including number and abundance of species and changes in time of the abundance of NIS introduced (<https://serc.si.edu/research/projects/large-scale-surveys-fouling-zooplankton-and-soft-sediment-benthic-habitats>; accessed July 2021).



**Figure 2.3 PVC panel attached to a brick (as weight) and to a rope: this is the standardized experimental unit of the SERC protocol.**

Many studies were conducted with this methodology all over the world (see also de Rivera et al. 2005, Schloeder et al. 2013, Gartner et al. 2016, Simkanin et al. 2016, Bastida-Zavala et al. 2017, Marraffini et al. 2017, Sun et al. 2017, Tracy et al. 2017, Jurgens et al. 2018, Chang et al. 2018, Newcomer et al. 2018, 2019, Carlton 2019, Torchin et al. 2021, Tamburini et al. 2021), and in the framework of this thesis it was applied for the first time in the Mediterranean Sea.

More procedural details on this protocol can be found in the paragraph 3.3. Moreover, these experimental units can be used also for manipulative experiments, as shown in thesis, where panels were transplanted from a site to another one for testing “biotic resistance hypothesis” (Aim 2, see

chapter 4) or panels were caged to assess the role of predation in the development of fouling assemblages, with a focus on NIS (Aim 3, see chapter 5).

### **3. Aim 1: Monitoring fouling communities through the SERC protocol**

This chapter is largely based on the paper: “Monitoring Non-indigenous Species in Port Habitats: First Application of a Standardized North American Protocol in the Mediterranean Sea” by Tamburini et al. (2021).

#### **3.1 Background**

Maritime traffic plays a key role in the introduction of fouling NIS, favouring their first settlement and establishment in ports, and then creating a hub and spoke network for the continuous spreading of NIS. It has long been considered that large commercial ports act as primary hubs for fouling NIS, due to the transit of transoceanic cargo ships, while small boats are involved in their secondary spread in marinas and neighbouring areas (Minchin et al. 2006, Floerl et al. 2009, Hulme 2009, Ros et al. 2013, Zabin 2014, Marchini et al. 2015). On the other hand, recent studies have suggested the importance of recreational boating in the primary introduction and spread of NIS (Ashton et al. 2014, Ferrario et al. 2017, Ulman et al. 2019a b). After introduction to a port environment, the settlement of NIS can be facilitated both by the large abundance of available artificial substrates (Glasby et al. 2007) and the tolerance of these species to different environmental conditions, including polluted waters (Piola & Johnston 2008, Piola et al. 2009).

Ports and marinas can be considered ideal sites for the early detection and monitoring of NIS presence and abundance, due to their importance as hotspot areas of NIS (Olenin et al. 2011, Ojaveer et al. 2014, Marraffini et al. 2017). However, no formal protocol has yet been adopted, and not even proposed, for the standardized assessment of fouling NIS in the European and Mediterranean countries. Monitoring of marine fouling communities is fundamental to track the introduction and spread of NIS, as well as to evaluate the power of legislation designed for limiting further introductions (Ruiz & Hewitt 2002, Lehtiniemi et al. 2015, Marraffini et al. 2017). The implementation of long-term monitoring programs and a preventive approach can contribute to the assessment of the potential risks of NIS, that have been demonstrated to negatively affect native communities - e.g., contributing to the decrease of vulnerable species (Marraffini et al. 2017, Occhipinti-Ambrogi 2021). For these reasons, a standardized monitoring and a coordinated program among countries should be recommended, in order to get comparable data across time and space (Hewitt & Martin 2001, Olenin et al. 2010, 2011, Latombe et al. 2017, Bailey et al. 2020). This is also in accordance with the Marine Strategy Framework Directive (MSFD 2008/56/EC), which aims to promote the development and application of standardized methods to assess the status of marine

coastal habitats, including the detection of NIS – one of the descriptors included in the assessment of the Good Environmental Status in European marine waters.

### **3.1.1 Monitoring methods of fouling communities and NIS in ports: the importance of long-term monitoring and cost-benefit analysis of current methodologies**

Several methodologies for sampling fouling communities – including NIS - in port habitats have been developed over the years. Most common techniques include I) Rapid Assessment Surveys – RAS (e.g. Cohen et al. 2005, Campbell et al. 2007, Olenin et al. 2007, Minchin 2012, Lehtiniemi et al. 2015, Marchini et al. 2015, Ulman et al. 2017, Gewing & Shenkar 2017); II) net-assisted scraping (e.g. Ulman et al. 2017, Ferrario et al. 2017, Tempesti 2020), including quadrat scraping during snorkelling or scuba diving (e.g. Neves et al. 2007, Awad et al. 2014, Ulman et al. 2019b); III) Artificial Substrate Units (ASU), such as two-dimensional settlement panels (e.g. Relini 1977, Canning-Clode et al. 2013, dos Santos Schwan et al. 2016, Lezzi et al. 2017, Marasinghe et al. 2018, Leclerc & Viard 2018, Carlton 2019, Leclerc et al. 2019, Outinen et al. 2021) and three-dimensional artificial habitat collectors (e.g. Fowler et al. 2013, Danovaro et al. 2016, Cahill et al. 2018, Gestoso et al. 2019, Outinen et al. 2019, Obst et al. 2020, Ros et al. 2020, Holmes & Callaway 2020).

Any of the above methods involves both advantages and disadvantages, and their success or suitability depend on the aim of the study. These methodologies can be classified based on four main features: “cost-effectiveness” of the method; “rapidity” of sampling; “quantification” of identified taxa, that indicates if there is a quantitative estimation of taxa abundance in samples or not; and level of “comprehensiveness” of the whole assemblage (i.e., if sessile and/or mobile taxa are sampled through the technique) (Table 3.1). As examples, quantitative methods (e.g., quadrats, settlement panels) are useful to thoroughly assess the entire fouling community but require a lot of fieldwork and laboratory effort. Conversely, qualitative methods like RAS allow for the collection of a lot of data on species distribution in a short timeframe, but the focus is typically only on target NIS already known and easily recognizable in the field. The latter method can limit the early detection of new arrivals, particularly of inconspicuous NIS. Net-assisted scraping probably ensures the most comprehensive picture of the fouling community and can provide both semi-quantitative (Ferrario et al. 2017) and quantitative outputs (Tempesti et al. 2022), yet it requires long laboratory analyses. In addition, some methods are more suitable for sessile taxa (e.g., settlement panels), while other ones are better for mobile taxa (e.g., scrapes, 3-D ASU). Light exposure and orientation of the ASU also affect the ability of collecting the photosynthetic components of fouling communities. Therefore, the

best practice for comprehensive sampling fouling communities should be a simultaneous multi-method approach in the same study area (Tait et al. 2018, Kakkonen et al. 2019).

**Table 3.1 Pro and cons of the most common methodologies used for the monitoring of fouling communities.**

<b>Methodology</b>	<b>Cost-effectiveness</b>	<b>Rapidity</b>	<b>Quantification</b>	<b>Comprehensiveness</b>
RAS	yes	yes	no	no
Scraping net	yes	no	no	yes
Quadrat scraping	no	no	yes	yes
Settlement panels	yes	no	yes	no
Artificial substrate units	yes	no	yes	no
Collectors	yes	no	no	yes

Moreover, in recent times, approaches such the metabarcoding have also started to be employed in NIS surveys with satisfactory results (see also Borrell et al. 2017, Pearman et al. 2021, Miralles et al. 2021). Metabarcoding can be considered an efficient tool to detect cryptic species or early life stages, but the main disadvantages for an extended use are the incompleteness of genetic sequence reference libraries (Zaiko et al. 2018, Duarte et al. 2021) and difficulties in morphological identification (i.e. the taxonomic gaps, see also Holman et al. 2019, Fernandez et al. 2021). For these reasons, the gold standard for complete and exhaustive surveys should be an integrative approach (Padial et al. 2010, Cahill et al. 2018, Obst et al. 2020, Duarte et al. 2021).

### **3.1.2 NIS in EU and Mediterranean ports**

In recent years, several sampling efforts focused along European and Mediterranean ports were conducted to monitor NIS presence. Using RAS, Ashton et al. (2006) sampled the 10 largest marinas in Scotland and found from one to three NIS in seven of them; in the Northern part of the same country, Nall et al. (2015) found a total of 10 NIS in 31 ports, while Bishop et al. (2015) found a total of 14 NIS in 17 marinas in the English Channel. Several species were found among all these assessments, above all ascidians and bryozoans.

In two harbours of the Normandy region, Verlaque & Breton (2019) found 14 macroalgal NIS through scuba diving, involving citizen scientists after a multi-year field survey. In the ports of Gdynia and Gdańsk (Baltic Sea), Brzana et al. (2019) found a non-indigenous tanaid species using both settlement panels and artificial habitat collectors. In a touristic marina of the subtropical island of Madeira (Atlantic Ocean), Canning-Clode et al. (2013) found 16 NIS after a 6-year fouling survey through



settlement panels. Several of these NIS are commonly found also in the Mediterranean region, while others are considered native for the Mediterranean.

Regarding the Mediterranean Sea, in the Western sector Ferrario et al. (2017) found a total of 22 NIS in 10 ports (Sardinian Sea, Ligurian Sea and North Tyrrhenian Sea) by scraping the artificial hard substrates with a rigid hand-held net. Similarly, Tempesti et al. (2020) collected in the port area of Leghorn 26 NIS and cryptogenic species. In the Taranto Sea, just outside the commercial port of Taranto (Ionian Sea), Lezzi et al. (2017) reported 16 NIS after a two-year macrofouling monitoring program using PVC settlement panels. Along the Slovenian coasts, Fortič et al. (2019) combined different methods (RAS, scraping net and scuba dives) to find six non-indigenous bryozoans in different ports. In the Southern Mediterranean Sea, Chebaane et al. (2019) reported 22 NIS sampled through RAS from five fishing ports and one marina in Monastir Bay, Tunisia. Lastly, the broadest investigation of non-indigenous invertebrates in Mediterranean marinas was conducted by Ulman et al. (2017) with both modified RAS and scrapes by hand-held rigid net for a total of 76 NIS found in 34 marinas. In general, the Mediterranean region shows similar fouling NIS community across the Western basin, probably due to the naval routes among the touristic marinas, that contribute to the spreading of NIS and to the homogenization of fouling assemblages in the marinas (Ulman et al. 2019a).

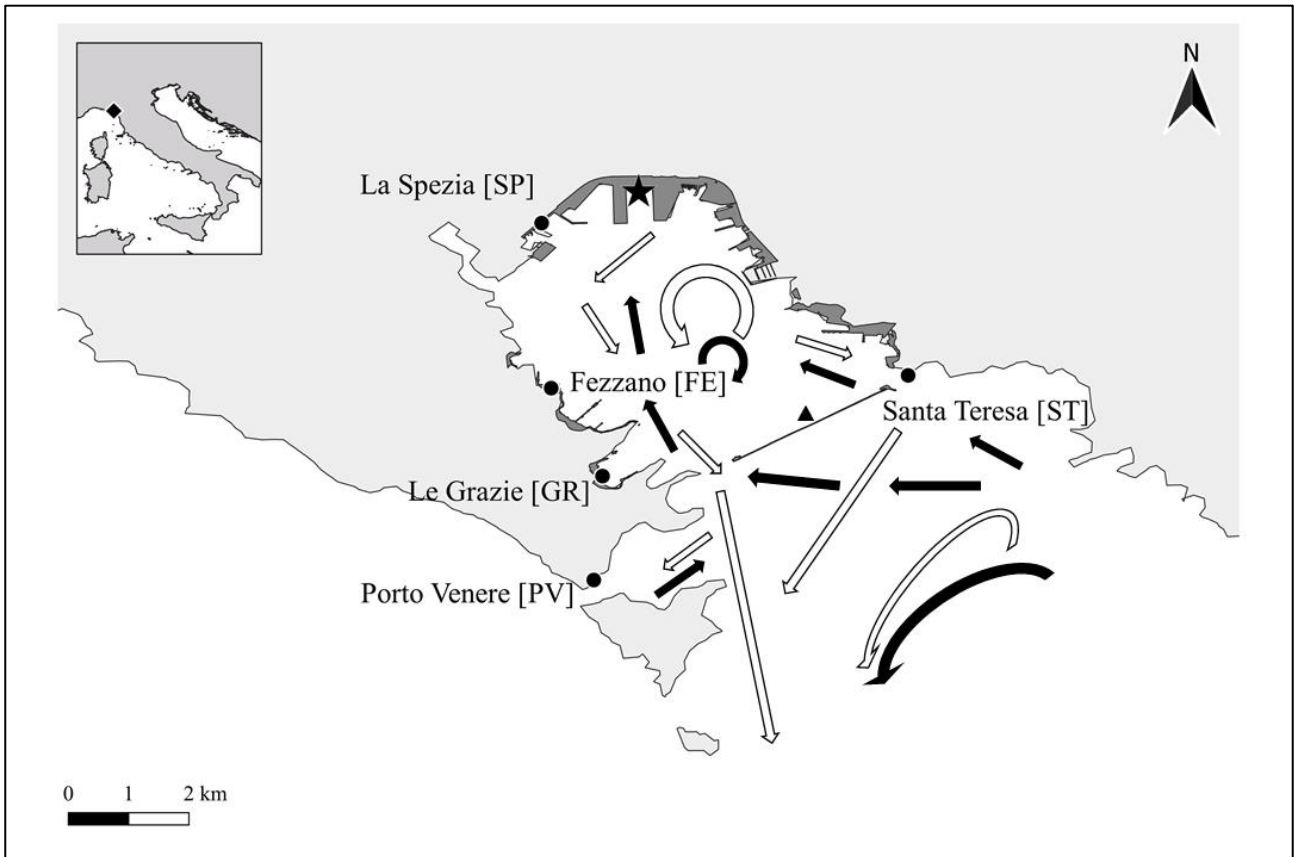
### **3.2 Aim of the study**

In this study it was applied for the first time in the Mediterranean Sea—namely in the Gulf of La Spezia (Ligurian Sea, Italy) — an international standard protocol for sessile fouling NIS monitoring in port habitats, with the aim to assess: (I) the fouling communities and NIS colonization in different harbour sites; (II) the development of NIS colonization in the fouling communities over time; (III) the effectiveness of this protocol for the early detection of newly introduced NIS in the Mediterranean; and (IV) the relationship between abundance of NIS and density of artificial structures in port habitats. Moreover, all results were shared with the MarineGEO network, a global network of partners led by the SERC focused on understanding how coastal marine ecosystems work, focusing on marine biodiversity (<https://marinegeo.si.edu/>).

### **3.3 Materials and methods**

The Gulf of La Spezia (44.09° N, 9.85° E) is one of the largest bays of the Ligurian Sea (Italy), characterized by the presence of several anthropogenic activities, as described in more detail in

paragraph 2.2.1. The trend of sea surface temperature (-0.915 m) from August 2018 to August 2020 was obtained by extrapolating data from loggers (ONSET HOBO WaterTemp V2) deployed in the gulf for other experimental purposes by ENEA Marine Environment Research Centre (Pozzuolo di Lerici, La Spezia). The fouling monitoring program (aim 1) included five sites inside the gulf (Figure 3.1; Table 3.2): in the marinas of Fezzano, Le Grazie and Porto Venere (FE, GR and PV respectively), near the commercial harbor of La Spezia (SP), and in a small marina near a mussel culture facility in Santa Teresa (ST).



**Figure 3.1** Map of monitoring sites in the Gulf of La Spezia (Ligurian Sea, Italy). Dark grey polygons indicate main port areas; black star indicates the commercial harbor of La Spezia; black triangle indicates the artificial dam; white and black arrows indicate surface and deep main currents of the Gulf, respectively (adapted from Gasparini et al., 2009).

The monitoring was carried out for three consecutive years (2018–2020) by applying a standardized protocol developed by the Smithsonian Environmental Research Center (Edgewater, MD, United States) that is being used to detect and track NIS in the U.S. as well as Canada, Ecuador, and Panama (see also de Rivera et al. 2005, Gartner et al. 2016, Simkanin et al. 2016, Marraffini et al. 2017, Tracy et al. 2017, Jurgens et al. 2018, Chang et al. 2018, Newcomer et al. 2018, 2019b, Carlton 2019, Torchin et al. 2021). A total of 50 PVC settlement panels ( $14 \times 14$  cm) were deployed in the summer of each year in the Gulf of La Spezia, allocating randomly 10 panels in each site. Specifically, the

passive collector (substrate) is composed of a settlement panel attached to a brick, with a sanded surface facing downward to minimize algal growth and facilitate invertebrate recruitment (Crooks et al. 2011, Marraffini et al. 2017, Jimenez et al. 2018). The experimental units were secured to docks or floating pontoons using rope, suspended 1 m below the sea surface, and immersed for three months during the summer season, in order to maximize the larval recruitment (Freestone et al. 2011, Marraffini et al. 2017). In 2018, the panels were immersed from July to October (87 days of deployment), in 2019 from April to July (89 days), and in 2020 from May to August (101 days). Differences in community assembly observational time across years were due to logistical constraints, in addition to mobility restrictions related to the health emergency of Coronavirus disease (COVID-19) and were considered during data analysis. At the end of the immersion period, all panels were retrieved, photographed, and observed under dissecting microscopes for taxonomic identification of sessile invertebrates. The percent cover of the whole fouling assemblage was assessed by point count method ( $n = 50$  points; Chang et al., 2018), using a plastic grid ( $7 \times 7$  points, with an additional 50<sup>th</sup> point chosen randomly) positioned upon the panels observed under a dissecting microscope. With this method it is possible to observe more than one taxon in the same counting point, therefore percent cover for the whole community may be exceed 100%. Fouling communities on panels were retrieved and observed in vivo in 2018, while in 2019 and 2020 the panels were preserved in 70% ethanol upon retrieval and stored for later analysis.

**Table 3.2 Geographical coordinates of the monitoring sites in the Gulf of La Spezia. Latitude and longitude are in the WGS84 datum. Abbreviations of sites are indicated in Figure 3.1.**

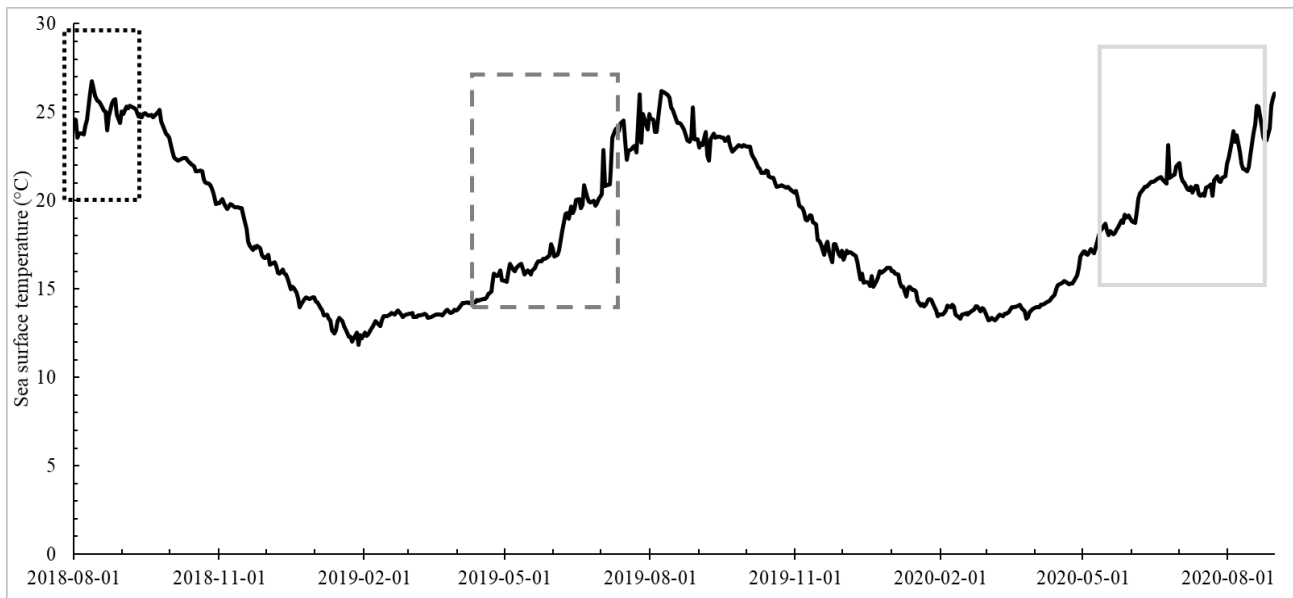
Site	Latitude (° N)	Longitude (° E)
FE	44.079643	9.828675
SP	44.104239	9.827251
GR	44.066539	9.836323
PV	44.051057	9.835024
ST	44.081544	9.881881

Species richness (S), NIS/native ratio and percent cover of each taxon were calculated for each panel ( $N = 10$  per site). S and percent cover were assessed separately for all taxa, together with the assignment of biogeographic status (sensu Chapman & Carlton 1991): NIS, native (including cryptogenic species—i.e., species that are not discernibly native or introduced, as defined by Carlton, 1996) and unresolved status (due to insufficient taxonomic resolution e.g., damaged specimens or juveniles). Differences in mean values for richness and percent cover of native (including cryptogenic

species) and NIS were assessed by two-way ANOVA (Type III tests with two orthogonal fixed factors: “year,” three levels: 2018, 2019, 2020; and “site,” five levels: FE, GR, PV, SP, ST; unequal sample size). When pooling across sampling years, differences in mean richness and percent cover of NIS were assessed by one-way ANOVA (fixed factor “site,” five levels: FE, GR, PV, SP, ST; unequal sample size). In case of non-homogeneity of the variances, data were properly transformed. A more conservative critical value ( $\alpha < 0.01$ ) was chosen if the data transformation was not successful. Tukey’s tests were used for post hoc comparisons of means. Percent cover data were analysed using principal coordinate analysis (PCO) in order to visualize the pattern of clustering among fouling communities from each site during the years of monitoring. The ordination technique was carried out on centroids (year  $\times$  site), based on Bray-Curtis similarity on fourth root transformed taxa percent cover data. PCO both for the whole community, and for NIS and native species component were applied separately. Distance-based test for homogeneity of multivariate dispersions (PERMDISP) and permutational multivariate analysis of variance (PERMANOVA) were carried out to test differences in NIS percent cover values of communities among sites (Anderson et al. 2008). Time was not considered as factor in this analysis, due to the different deployment period among years, that did not allow a fair comparison. Correlation between NIS percent cover and density of immersed artificial structures (e.g., docks, pontoons) of each monitoring site was tested, as suggested by Susick et al. (2020). Density of each site was measured through satellite images as the linear length of mooring structures within a 200 m diameter circle of the potential marine habitat, centred on the spatial centroid of deployed panels. Length of artificial structure visible above water was measured using the path ruler tool in Google Earth Pro v.7.3.3.7699 (Susick et al. 2020). Analyses were carried out with the software Microsoft Excel, R (R Core Team 2017), QGIS (QGIS Development Team 2020) and PRIMER 6 with PERMANOVA C add-on package (Clarke & Gorley 2006, Anderson et al. 2008).

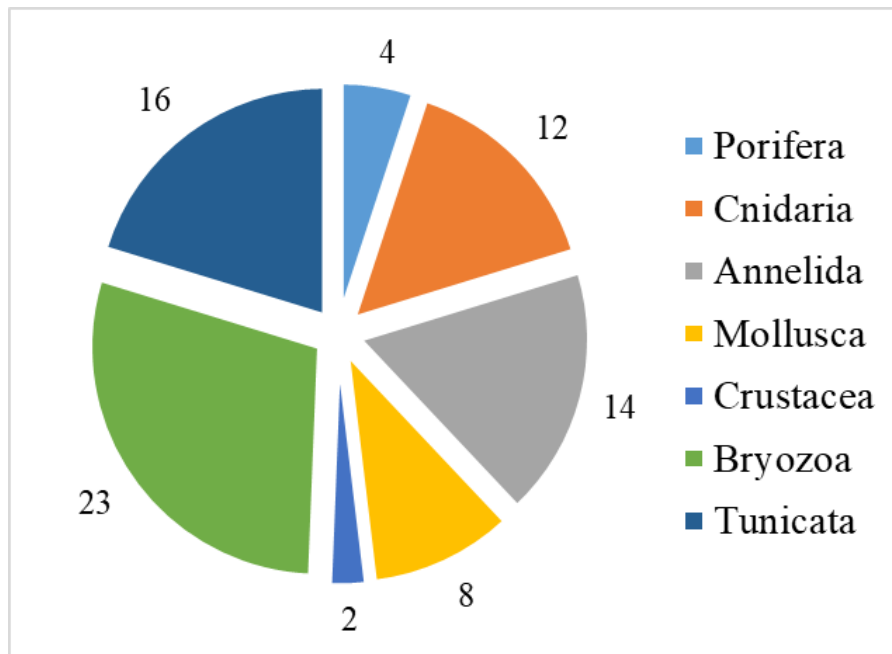
### **3.4 Results**

From August 2018 to August 2020, the range of surface temperature varied from 12° to 27°C (Figure 3.2). Mean temperatures during the monitoring periods were 25°±1°C in 2018, 19°±3°C in 2019 and 21°±2°C in 2020. Overall, 126 out of the 150 panels initially deployed in the three years were collected; the remaining panels (nine in 2018, four in 2019, and 11 in 2020) were lost due to external causes (e.g., coastal storms or vandalism).



**Figure 3.2** Extrapolated trend of surface temperature (-0.915 m), starting from data obtained by loggers deployed in the Gulf for other purposes (ENEA, La Spezia). Over-imposed grey rectangles indicate the panel deployment periods during the three-year monitoring.

After the 3-year monitoring, a total of 79 sessile taxa representing seven phyla were documented from the Gulf of La Spezia (Figure 3.3; Supplementary table 1), including 11 NIS: four Bryozoa, four Annelida, one Porifera, and two Tunicata (Table 3.3). In the Table 3.3 is also shown the mean NIS cover in sites and years. Among all the NIS observed, one has not been identified at species level (i.e., *Branchiomma* sp.). This species has been firstly assigned to *Branchiomma boholense* (Grube, 1878), but an accurate analysis of the specimens revealed divergent taxonomic characters, that cannot be attributed to the other *Branchiomma* spp. known to occur in the Mediterranean Sea, including the non-indigenous *Branchiomma bairdi* (McIntosh, 1885), *B. boholense* and *Branchiomma luctuosum* (Grube, 1870). Thus, further analyses are needed to confirm its identity, which could also be assigned to a fourth NIS belonging to the genus *Branchiomma*.



**Figure 3.3** Number of taxa found in the Gulf of La Spezia grouped by taxonomic macrogroups.

The majority of the NIS were detected during all three monitoring campaigns, with the exception of *Botrylloides cf. niger* Herdman, 1886 - found only in 2018 - and *B. luctuosum*, found in 2018 and 2020. NIS were detected on all panels sampled over 3 years, except for a single panel at PV in 2019. Furthermore, nine panels exhibited only one NIS, seven of which were collected in PV from 2018 to 2020.

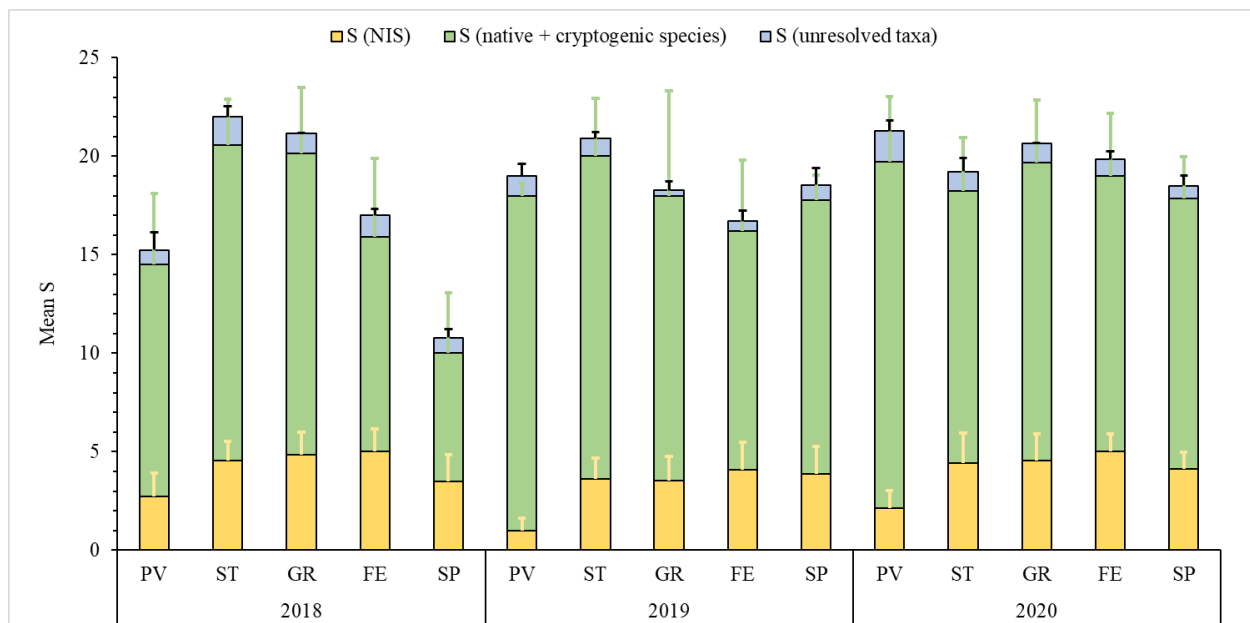
**Table 3.3** List of the NIS found in the investigated localities. The mean cover of each NIS in sites and years is shown. Asterisk indicates NIS found for the first time in the Gulf of La Spezia.

Phylum	Species	Fezzano (FE)			Le Grazie (GR)			Porto Venere (PV)			La Spezia (SP)			Santa Teresa (ST)		
		2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020
Porifera	* <i>Paraleucilla magna</i> Klautau, Monteiro & Borojevic, 2004	1%	0.2%	0.2%	3%	0.1%	0.3%	1%	0%	1%	2%	0%	0%	6%	0.2%	2%
Bryozoa	<i>Amathia verticillata</i> (Delle Chiaje, 1822)	7%	1%	0.0%	0%	0.1%	0%	0%	0%	0%	24%	19%	30%	0.1%	0.1%	2%
	<i>Celleporaria brunnea</i> (Hincks, 1884)	2%	8%	7%	2%	4%	4%	0.1%	0%	0%	2%	5%	16%	0%	0.2%	0.1%
	<i>Tricellaria inopinata</i> d'Hondt & Occhipinti Ambrogi, 1985	1%	10%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	11%	52%	7%
	* <i>Watersipora arcuata</i> Banta, 1969	0.2%	0.2%	0%	0.2%	0%	0.1%	0.1%	0%	0.4%	0.1%	0.2%	0.1%	0.3%	0%	0%
Annelida	* <i>Branchiomma</i> sp.	0.1%	1%	1%	0%	1%	1%	0%	1%	1%	0%	0.4%	0.1%	1%	1%	1%
	* <i>Branchiomma luctuosum</i> (Grube, 1870)	0.4%	0%	1%	1%	0%	1%	0.4%	0%	0%	0.1%	0%	3%	0%	0%	0.3%
	<i>Hydroides dirampha</i> Mörch, 1863	1%	0.1%	11%	1%	0%	5%	1%	0%	0%	5%	0.2%	12%	1%	0%	1%
	<i>Hydroides elegans</i> (Haswell, 1883)	98%	24%	20%	91%	41%	18%	36%	3%	2%	86%	25%	20%	74%	8%	1%
Tunicata	* <i>Botrylloides cf. niger</i> Herdman, 1886	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.1%	0%	0%
	<i>Styela plicata</i> (Lesueur, 1823)	1%	7%	4%	2%	3%	4%	0%	0%	0.1%	0.1%	1%	2%	0%	4%	6%

The highest values of NIS richness ( $S_{NIS} = 7$ ) found on single panels were from FE 2018, GR 2020, and ST 2020. For species richness, the lowest value of mean S at each site, i.e.,  $S_{tot} (\pm SD)$ , was

observed in SP in 2018 ( $11 \pm 4$ ), while the highest in ST in the same year ( $22 \pm 2$ ) (Figure 3.4). Regarding the mean  $S_{NIS}$ , the lowest value was recorded in PV in 2019 ( $1 \pm 1$ ) and the highest in FE, GR and ST in 2018, as well as in FE and GR in 2020 (about five species on average).  $S$  of native and cryptogenic species ( $S_{native}$ ) was the lowest in SP 2018 ( $7 \pm 3$ ) and the highest in PV 2020 ( $18 \pm 3$ ; Figure 3.4). The mean  $NIS/(native + cryptogenic \text{ species})$  ratio showed a similar pattern, registering the lowest values in PV 2019 and 2020 ( $0.1 \pm 0.04$ ) and the highest in SP 2018 ( $0.6 \pm 0.2$ ).

$S_{native}$  resulted significantly different among sites, but not across years ( $F = 11.3172, p = 9.73 \times 10^{-8}$ ;  $F = 1.6857, p = 0.19003$ , respectively). The site  $\times$  year interaction resulted significant ( $F = 3.9884, p = 3.462 \times 10^{-4}$ ) and post hoc test revealed that a single site (SP) contributed to the difference in interaction (2018 vs. 2019 adjusted  $p = 2.913 \times 10^{-4}$ ; 2018 vs. 2020 adjusted  $p = 7.745 \times 10^{-4}$ ). Similarly,  $S_{NIS}$  showed significant differences only among sites ( $F = 5.5382, p = 4.21 \times 10^{-4}$ ) and not across years ( $F = 1.7775, p = 0.1738433$ ), and no significant interaction was detected ( $F = 0.988, p = 0.4493411$ ). Among the sites, PV showed significant differences with all the other four sites (adjusted  $p < 0.001$ ).



**Figure 3.4 Mean species richness (S + standard deviation) of each monitoring site during the years of monitoring. Sites are showed at decreasing distance from La Spezia (SP). Abbreviations of sites are indicated in Figure 3.1.**

Merging the data of the three years of monitoring, the sites with the highest and the lowest values of mean  $S_{tot}$  were ST ( $21 \pm 3$ ) and SP ( $16 \pm 5$ ), respectively; while considering  $S_{NIS}$ , the highest value was found in FE ( $5 \pm 1$ ) and the lowest in PV ( $2 \pm 1$ ). Most of the sites displayed higher values of mean  $S_{native}$ , namely GR ( $15 \pm 4$ ), PV ( $15 \pm 4$ ), and ST ( $15 \pm 3$ ); conversely, SP ( $11 \pm 4$ ) showed a slightly lower value (Table 3.4).

**Table 3.4 Mean values of S ( $\pm$ SD) per site for the entire three-year period of monitoring. S (total species): mean number per plate of taxa in each site; S (NIS): mean number per plate of NIS in each site; S(native + cryptogenic species): mean number per plate of native and cryptogenic species in each site. Abbreviations of sites are explained in Figure 3.1.**

<b>Site</b>	<b>S (total species)</b>	<b>S (NIS)</b>	<b>S (native + cryptogenic species)</b>
FE	17.5 $\pm$ 4.4	4.7 $\pm$ 1.2	12.1 $\pm$ 3.7
GR	19.8 $\pm$ 4.9	4.2 $\pm$ 1.3	14.9 $\pm$ 4.1
PV	18.3 $\pm$ 4.4	2.0 $\pm$ 1.2	15.2 $\pm$ 4.0
SP	15.7 $\pm$ 4.7	3.8 $\pm$ 1.2	11.1 $\pm$ 4.2
ST	20.6 $\pm$ 3.0	4.2 $\pm$ 1.3	15.4 $\pm$ 2.9

During the three years of monitoring, mean percent cover of NIS was the lowest in PV (17 $\pm$ 23%) and the highest in SP (85 $\pm$ 50%). Mean percent cover of native and cryptogenic species (Figure 3.5A) was highest value in GR 2020 (141 $\pm$ 32%) and lowest in SP 2018 (42 $\pm$ 35%). For NIS, mean percent cover (Figure 3.5B) was highest in SP 2018 (118 $\pm$ 38%) and lowest in PV 2019 (4 $\pm$ 5%). Percent cover of native species was significantly different across years ( $F = 9.0727$ ,  $p = 2.241 \times 10^{-4}$ ) and sites ( $F = 3.1586$ ,  $p = 0.0168232$ ), with no significant interaction ( $F = 1.5418$ ,  $p = 0.1508879$ ). Across years, the values observed in 2020 showed significant differences with 2018 and 2019 (adjusted  $p < 0.001$ ); among sites, PV resulted significantly different from FE and SP (adjusted  $p < 0.01$ ; Figure 3.5A). Significant main effects were detected for NIS cover among sites ( $F = 7.5473$ ,  $p = 2.05 \times 10^{-5}$ ) and across years ( $F = 14.5618$ ,  $p = 2.42 \times 10^{-6}$ ), but no interaction effect was detected ( $F = 1.2237$ ,  $p = 0.2918$ ). Post hoc tests revealed that percent cover of NIS was different between 2018 and the other two years ( $p < 0.001$ ); between PV and the other four sites ( $p < 0.01$ ); between ST and the other sites ( $p < 0.01$ ; Figure 3.5B).

The investigated sites were significantly different in terms of mean richness and percent cover of NIS, considering the three years of monitoring ( $F = 14.691$ ,  $p = 8.257 \times 10^{-10}$ ;  $F = 13.494$ ,  $p = 4.00 \times 10^{-9}$ , respectively). Tukey's tests showed a significant difference in richness between PV and all the other sites ( $p < 0.001$ ) when considering  $S_{NIS}$ . On the basis of NIS cover a significant difference came out between the sites PV-FE, PV-GR, and PV-SP ( $p < 0.001$ ); ST-FE ( $p < 0.05$ ) and ST-SP ( $p < 0.001$ ). Relative abundance - i.e., percent cover data standardized to 100% - of unresolved taxa resulted very low in all sites (1-2%); while values for NIS and native species were similar in FE and SP (about 50% each); and in GR, PV, and ST the relative abundances were higher for the native species (63%, 85%, and 71%, respectively) in comparison with the NIS (36%, 13%, and 28%, respectively; Figure 3.6).



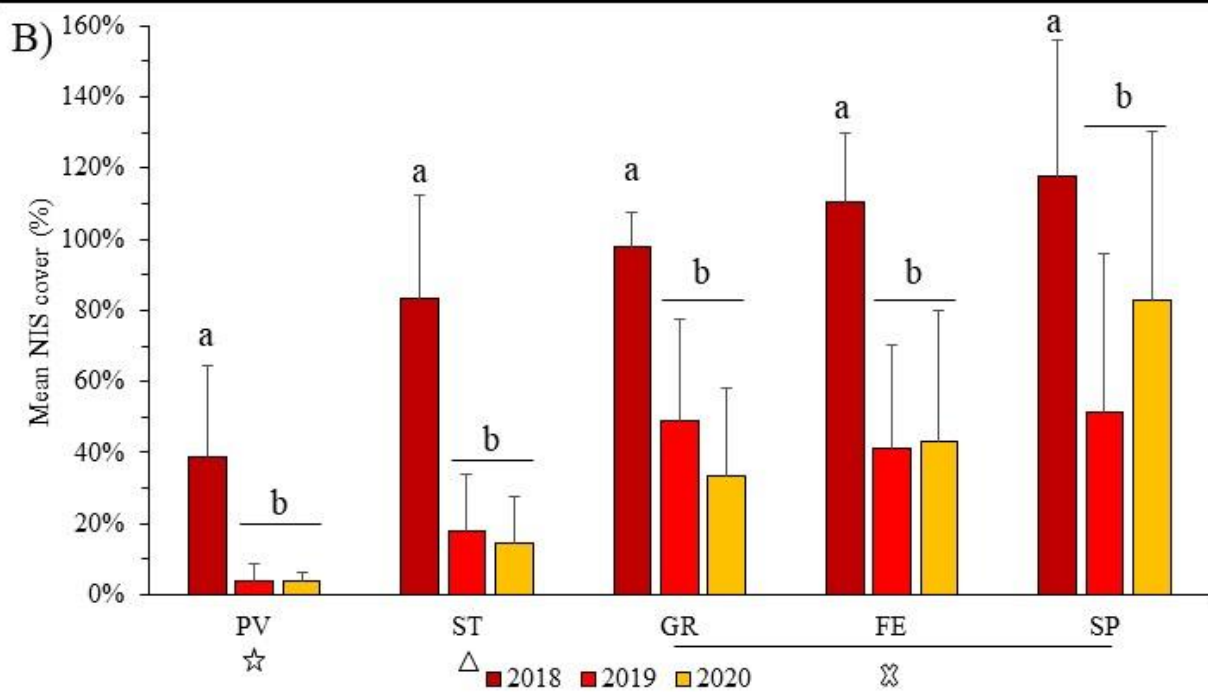
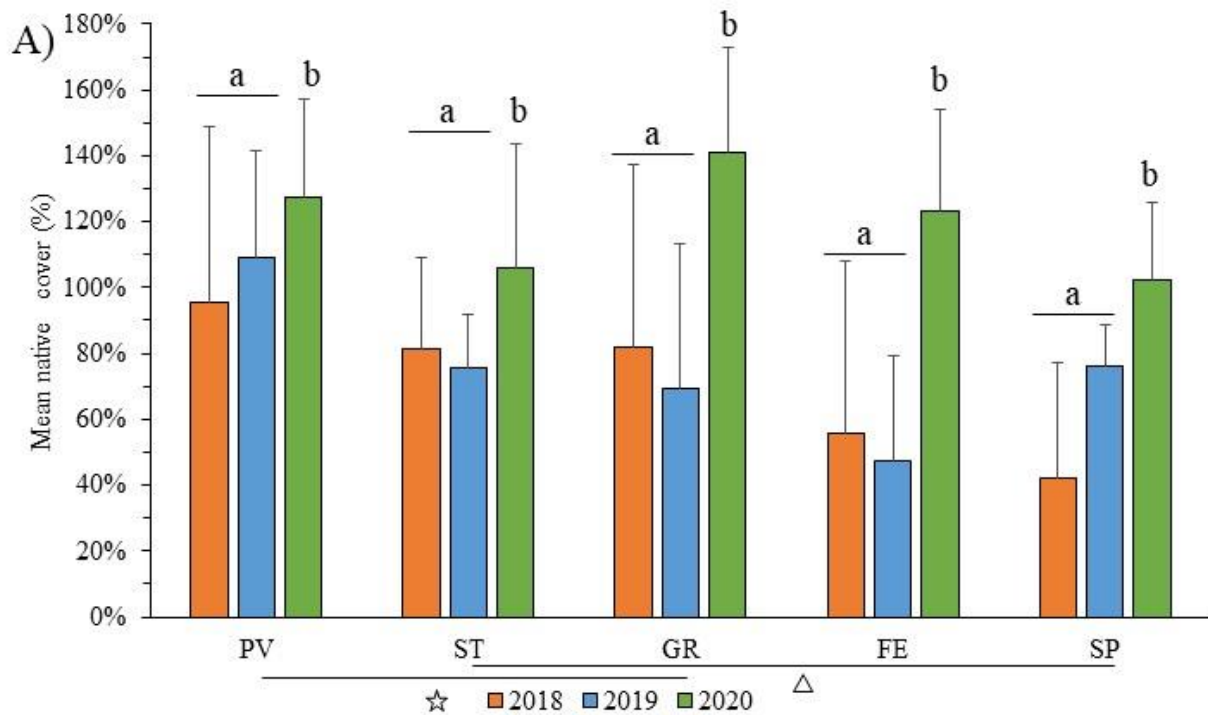
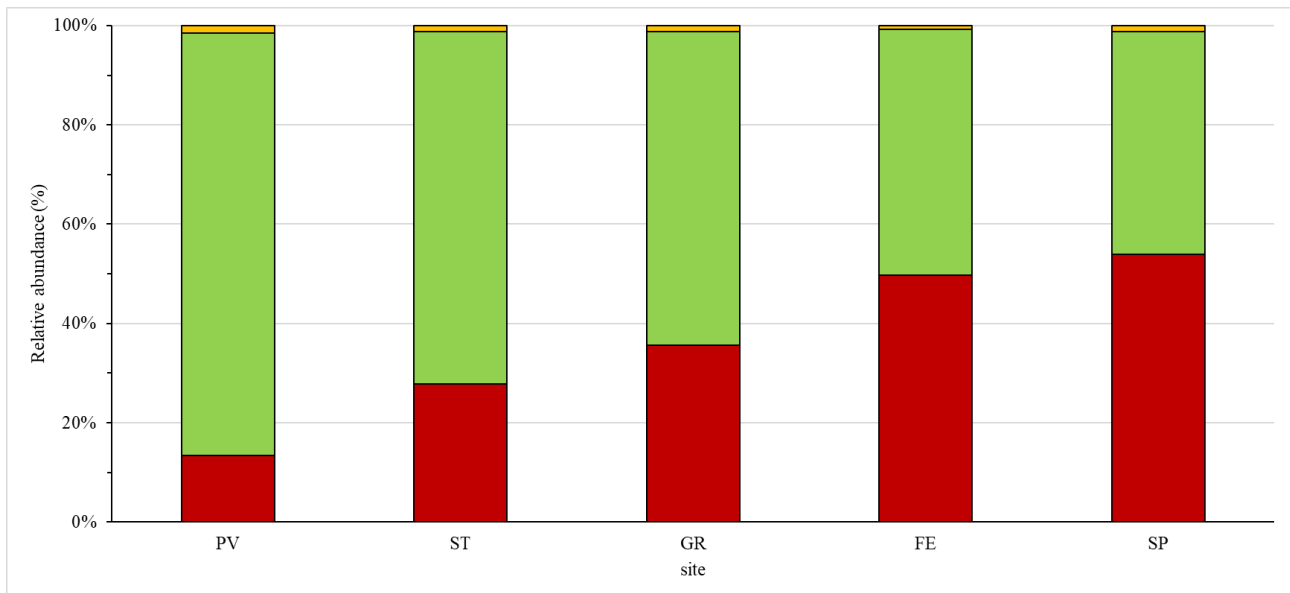


Figure 3.5 Mean (+SD) percent cover of native and cryptogenic species (A), and NIS (B) in each monitoring site during the three years of monitoring. Letters (a, b) on the top of the two graphs indicate statistical divergence among years, while symbols on the bottom indicate statistical divergence among sites. Sites are showed at decreasing distance from La Spezia (SP) and abbreviations of sites are indicated in Figure 3.1.



**Figure 3.6** Relative abundance (%) of NIS percent cover (red bar), percent cover of native and cryptogenic species (green bar) and unresolved taxa percent cover (orange bar) in the monitoring sites in the three-year monitoring. Sites are shown at decreasing distance from La Spezia (SP). Abbreviations of sites are indicated in Figure 3.1.

Regarding the structure of fouling communities, PCO showed slightly separated clusters per sampling year and the same distribution of sites within each year, with SP and PV being the most diversified sites (Figure 3.7). This pattern probably follows the percent cover of native species (Figure 3.8B) rather than NIS, which did not show a clear arrangement (Figure 3.8A): the values on percent cover of the non-indigenous component were more clumped than the native one (Figure 3.8). PERMANOVA main test on NIS percent cover data of communities among monitoring sites (years merged) showed significant dissimilarity among community composition [pseudo-F = 13.138,  $p(\text{perm}) = 0.0001$ , unique permutations: 9915], while PERMDISP highlighted significant deviations of samples from centroid [ $F = 3.7702$ ,  $p(\text{perm}) = 0.0116$ ], rejecting the hypothesis of no difference in dispersion among the groups, i.e., “non-homogeneity” of samples dispersion. Moreover, pairwise PERMANOVA tests showed significant differences among sites, except for FE and GR [ $t = 1.3337$ ,  $p(\text{perm}) = 0.1371$ , unique permutations: 9956], which therefore showed not-dissimilar NIS communities.

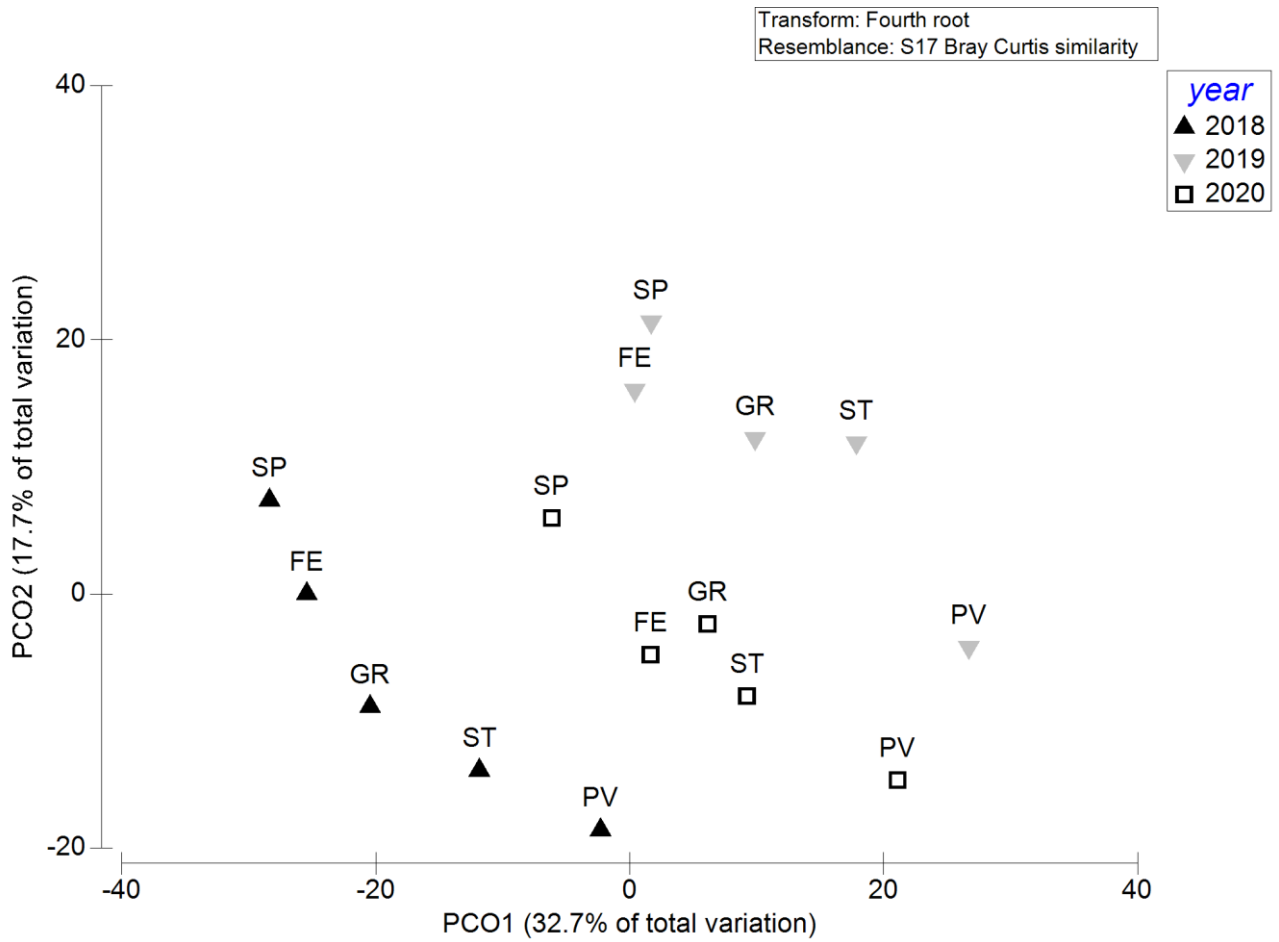
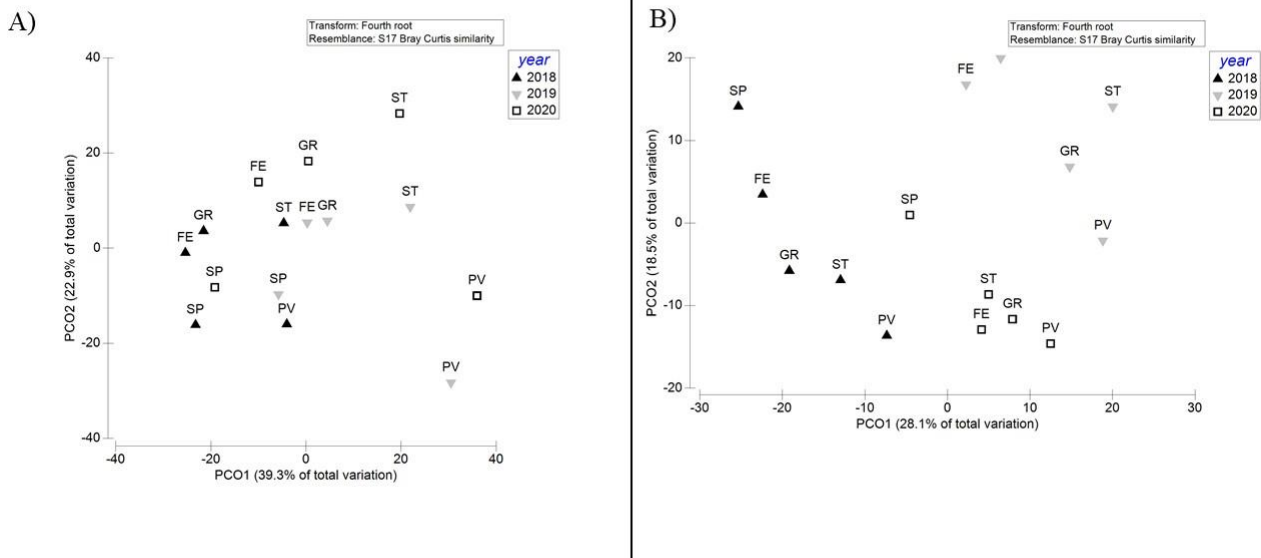
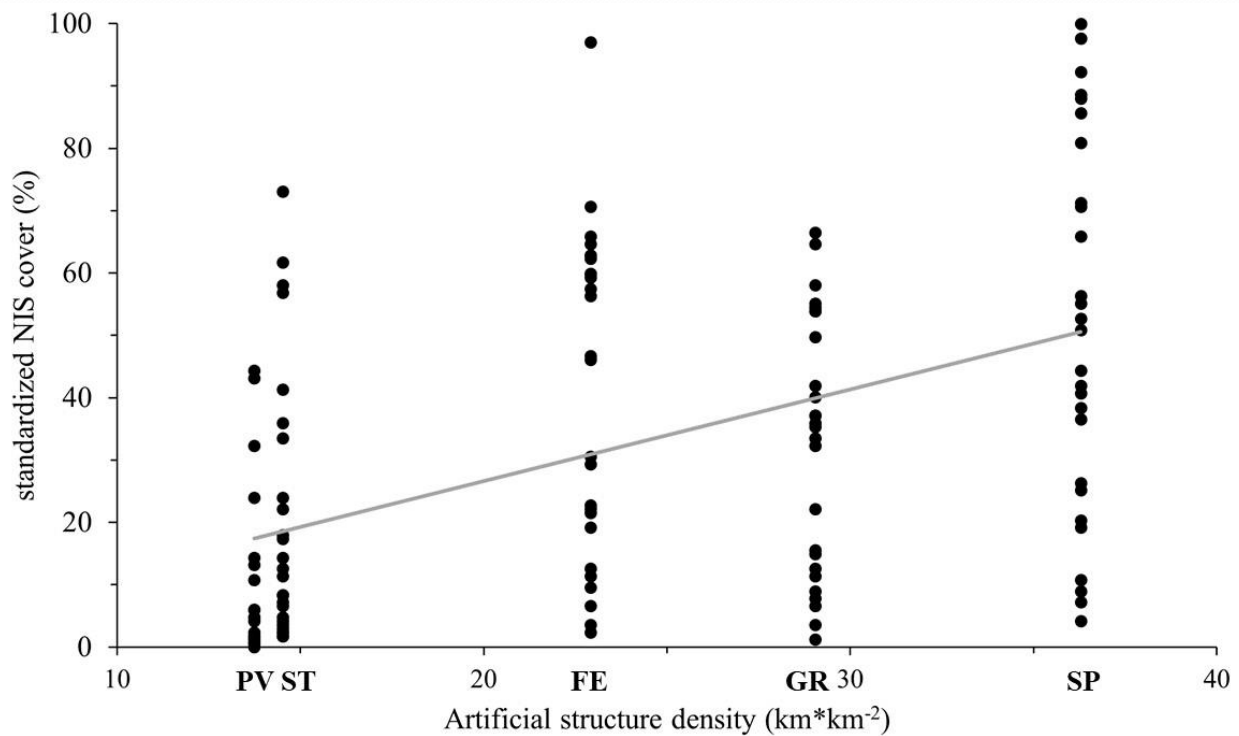


Figure 3.7 PCO (Principal Coordinate Analysis) based on distance among centroids (year x site) of whole fouling community percent cover data. Abbreviations of sites are indicated in Figure 3.1.



**Figure 3.8** PCO (Principal Coordinate Analysis) based on distance among centroids (year x site): (A) NIS percent cover data, (B) Native and cryptogenic species percent cover data. Abbreviations of sites are indicated in Figure 3.1.

Density of artificial structures was the highest in SP ( $36.28 \text{ km} \times \text{km}^{-2}$ ) and the lowest in PV ( $13.74 \text{ km} \times \text{km}^{-2}$ ), with intermediate values in GR ( $29.04 \text{ km} \times \text{km}^{-2}$ ), FE ( $22.91 \text{ km} \times \text{km}^{-2}$ ), and ST ( $14.51 \text{ km} \times \text{km}^{-2}$ ). Correlation between NIS percent cover of samples (126) and these calculated densities resulted low (adjusted  $R^2 = 0.2144$ ,  $p = 2.876 \times 10^{-8}$ ; Figure 3.9).



**Figure 3.9** Correlation of standardized NIS cover and artificial structure density of sites (n= 126, adjusted  $R^2=0.2144$ ,  $p=2.876 \times 10^{-8}$ ). Each black point represents the value of NIS cover on a panel. Abbreviations of sites are indicated in Figure 3.1.

### 3.5 Discussion

First of all, the extrapolated trend of surface temperature showed a strong seasonality during the years, with similar temperature trends during the 2019 and 2020 monitoring periods and slightly higher temperatures during 2018 monitoring period, carried out later (July-October).

The 3-year monitoring on fouling communities in the Gulf of La Spezia allowed us to collect 79 taxa, half of which belong to Bryozoa and Tunicata, and to detect a total of 11 NIS. Seven NIS were already reported in the Gulf in 2010–2013 (Lodola et al. 2012, 2015, Ferrario et al. 2017, Katsanevakis 2020) while five are new records, namely the sponge *Paraleucilla magna* Klautau, Monteiro and Borojevic, 2004, the sabellid polychaetes *Branchiomma* sp. and *B. luctuosum*, the bryozoan *Watersipora arcuata* Banta, 1969 and the ascidian *Botrylloides* cf. *niger*. Interestingly, in this study three NIS from Ferrario et al. (2017), observed in the harbor of La Spezia or in the marina of Lerici (near Santa Teresa site), were not recorded during the three years of monitoring, namely *Amphibalanus improvisus* (Darwin, 1854), *Arcuatula senhousia* (Benson, 1842), and *Ficopomatus enigmaticus* (Fauvel, 1923). This result could maybe be due to the recruitment and settlement of these NIS in more mature fouling assemblages, as those analysed by Ferrario et al. (2017).

For the NIS observed for the first time in La Spezia in the present study, *Paraleucilla magna* is the only sponge reported as non-indigenous in the Mediterranean Sea (Longo et al. 2007). It was first described in Brazil and its putative native origin is the Indo-Pacific region and Australia, while its current distribution in the Mediterranean includes Italy, Spain, Malta, Croatia, Greece, Turkey, and Cyprus (Ulman et al. 2017). Although *P. magna* was already reported in the Ligurian Sea, namely along the Portofino coasts (Bertolino et al. 2013, Longobardi et al. 2017), this study provides a new record for the Gulf of La Spezia. The hypothesis expressed by Bertolino et al. (2013) and Longobardi et al. (2017) on the potential expansion of this species from the mussel farms of the Gulf of La Spezia to Portofino area, is strengthened by this last finding. The highest mean cover of *P. magna* was indeed found in Santa Teresa, the monitoring site near aquaculture facilities. Regarding *Branchiomma* sp., we think this taxon must be further investigated, as this genus showed intraspecific variability that can lead to species misidentification (Capa et al. 2013). For example, a recent taxonomic study suggested that most of the Mediterranean specimens previously identified as *B. bairdi* should probably be considered as *B. boholense*, with the exception of data reported by Ulman et al. (2019a) in different Mediterranean countries (Del Pasqua et al. 2018, Langeneck et al. 2020). *Branchiomma luctuosum*, originally described from the Red Sea (Grube, 1870), was found for the first time in the Mediterranean Sea in Italy in 1978 (Phyllis et al. 1991). Then, *B. luctuosum* was recorded in several localities in the whole Mediterranean Sea, where it is considered established and able to colonize both artificial and natural substrates (Giangrande et al. 2012, Fernández-Romero et al. 2021). In the Western Mediterranean, *B. luctuosum* was found along the Spanish coasts since 2004 (El Haddad et al. 2008, López & Richter 2017), in the Gulf of Genoa in 2011-2015 (Bianchi et al. 2018), in Sardinia in 2014 (Ferrario et al. 2017, Langeneck et al. 2020), and more recently in Leghorn in 2016 (Langeneck et al. 2020, Tempesti et al. 2020); the latter locality being about 37 nautical miles away from La Spezia. The bryozoan *Watersipora arcuata* was reported for the first time in the Mediterranean Sea in 2013 in the Ligurian Sea (Ferrario et al. 2015). This species, likely originating from the Tropical Eastern Pacific, has now been recorded in most Mediterranean regions, namely Spain, Italy, Malta, and Turkey (Ulman et al. 2017, Ferrario et al. 2018). Furthermore, a recent revision of historical samples revealed its presence along the South Atlantic coast of Spain since 1990, suggesting its pathway of introduction in the Mediterranean Sea through the Gibraltar Strait (Reverter-Gil & Souto 2019). Finally, *Botrylloides niger*, a colonial ascidian native from the tropical Western Atlantic, is reported in several tropical and warm water regions across the world (Sheets et al. 2016), including Madeira island (Ramalhosa et al. 2021). In the Mediterranean Sea, it was first found in 2006 in Israel (Rubinstein et al. 2013) and erroneously identified as *Botrylloides leachii* (Savigny, 1816) (Griggio et al. 2014). In general, species in the genus *Botrylloides* are easily confused

(Brunetti 2009, Viard et al. 2019), and in absence of molecular analyses, a conservative approach by considering the identification as tentative was used, pending further verification.

Our monitoring showed that mean NIS richness varied from one to five species among sites and years, with similar values across the years of monitoring. For this reason, a clear increasing pattern in new NIS detections in the different sites was not found, but continuation of this monitoring is required to identify more robust temporal patterns. On the other hand, native species richness significantly varied only in La Spezia among years, highlighting a stable number of native species during the monitoring. It should be highlighted that *B. cf. niger* was recorded only in 2018 in ST, but in the following years it did not disappear from the marina. Despite not being found on panel communities, it was still observed, even if in very low abundances, on other artificial substrates (e.g., on the supporting structure of the panels and on floating pontoons; authors personal observation). For this reason, *B. cf. niger* may be considered as an established NIS in the study area. After 3 years of monitoring, Porto Venere was found to be the least impacted site in terms of both richness and percent cover of NIS, while the other sites showed a higher impact by NIS, with values of mean NIS percent cover ranging from 34% (Santa Teresa) to 85% (La Spezia).

In general, the different submersion periods - mid-summer in 2018 and early spring in the following years - affected the development of fouling assemblages, mainly in terms of differences in taxa percent cover. NIS richness was indeed similar across the years of monitoring, while in 2018 definitely higher mean values of NIS cover were observed in all sites, compared to the other years of monitoring. The latter result is probably a consequence of the later submersion period of 2018, which led to the dominance on the panels of one NIS, namely *Hydroides elegans* (Haswell, 1883). A similar phenomenon with the dominance of one single species in fouling communities was also observed in marinas of South-eastern Brazil (dos Santos Schwan et al. 2016), and could be related to the seasonal reproductive peaks (Reinhardt et al. 2013, Lezzi & Giangrande 2018, Fortič et al. 2021). These preliminary results suggest that maintaining the monitoring program between May and July is advisable. In fact, the number of detected NIS resulted independent from the submersion period, but during the period May-July it is possible to better capture the entire spectrum of potential colonizers, avoiding the dominance of a single species. Considering the whole data set on NIS percent cover of the 3-year monitoring, higher values were found in La Spezia, with Fezzano, Le Grazie, Santa Teresa, and Porto Venere gradually showing lower values. The same gradient from La Spezia to Porto Venere was also observed when considering the structure of the entire community, and the gradient was probably more influenced by the composition of the native species than to the non-indigenous one. These patterns may reflect the different geographic features of the monitoring sites. In fact, the sites

are located at an increasing distance from the main harbor of La Spezia, one of the largest commercial ports in Italy (<https://www.assoporti.it/en/home/>; accessed March 2021). The harbor of La Spezia hosts international cargo ships and passengers' vessels, as well as local ferries. The distance of sites from the main harbor appears to be proportional to the mean NIS percent cover, while the proximity of Santa Teresa to the mussel farm showed higher abundances of *P. magna*, a NIS associated to aquaculture (Bertolino et al. 2013), as well as the exclusive presence of *B. cf. niger* in comparison with other sites (see figshare repository: <https://doi.org/10.6084/m9.figshare.14758383.v2>). In addition, the water circulation inside the Gulf is different in Porto Venere compared to the other sites, due to both the presence of the dam and the distance from the open sea (Figure 3.1; Gasparini et al. 2009). This circulation could advantage NIS larval dispersion and settlement inside the dam, where water exchange is surely lower than in Porto Venere (Gasparini et al. 2009). On the other hand, the density of artificial structures seems not to be a factor influencing NIS distribution in the area. The observed variability of NIS percent cover in the dataset can also be related to other features of sites, like the presence of floating pontoons, or the proximity to aquaculture facilities or to commercial harbours (Nall et al. 2015, Ulman et al. 2019a). All these aspects seem to affect the distribution of NIS and the composition of fouling communities in the Gulf of La Spezia. A regular data collection in the Gulf will allow us to examine in depth these results and better clarify the observed dynamics, but this first attempt should be considered in future management measures to prevent NIS introduction in the area, by implementing more rigid controls in sites closer to the harbor of La Spezia. Moreover, a longer data collection could also allow for obtaining limited statistical dispersion of samples within sites, in order to assess if difference in NIS percent cover in monitoring sites is purely due to the dissimilarity among sites and not to dispersion of samples (Anderson et al. 2008).

In conclusion, this monitoring allowed us to implement, for the first time in the Mediterranean Sea, an international standard protocol developed by the SERC to assess fouling communities and detect NIS. The number of detected NIS was in line with previous monitoring carried out in the Gulf of La Spezia and in other Ligurian port localities, but with additional new records for the area and three NIS no longer observed (Ferrario et al. 2017, Ulman et al. 2019a, Katsanevakis 2020). These results are consistent with the role of commercial harbours and recreational marinas as hot-spot sites for the introduction of NIS (Clarke Murray et al. 2011, Ros et al. 2013, Ferrario et al. 2017, Verlaque & Breton 2019), and the importance of continuous monitoring to detect changes in marine communities (Olenin et al. 2011, Ojaveer et al. 2014, Lehtiniemi et al. 2015). This monitoring protocol seems to be a strong and effective tool to control the presence and abundance of fouling NIS in the Mediterranean ports. Its main advantages are cost-effectiveness, ease of application and the capability to obtain quantitative results in a relatively short time. On the other hand, the effort for sampling and



analysing the panels is considerable. Finally, this protocol is predominantly focusing on the assessment of the sessile hard-bottom fauna, not providing complete quantitative data on the mobile component living on the panels ( see Vicente et al. 2021 ), as well as on organisms dwelling in soft bottoms (e.g., see Chatzinikolaou et al. 2018), that should ideally be also considered for future monitoring programs on NIS in port habitats. While this methodology provides a reliable option for sampling and monitoring fouling communities in the Mediterranean area, further analyses to test its effectiveness should be encouraged in other regional localities, together with a comparison to other fouling monitoring tools (e.g. ARMS; Obst et al. 2020), including the use of genetic approaches (e.g. Ardura & Planes 2017, Borrell et al. 2017, Marraffini et al. 2017, Cahill et al. 2018, Kakkonen et al. 2019, Obst et al. 2020, Duarte et al. 2021). Successful implementation in other localities could lead to the proposal of this method as an adopted standard for the assessment of marine bioinvasions in the Mediterranean. In fact, the application of the SERC method across the Mediterranean Sea could provide a simple option to gain standardized and quantitative data in a key invasion-prone habitat, allowing rapid assessment and one approach to evaluate changes in the structure of fouling communities, as it is being carried out along multiple coasts and countries in the Americas.

## **4. Aim 2: Testing the biotic resistance hypothesis in fouling communities**

This chapter is largely based on the paper: “Biotic resistance of native fouling communities to bioinvasions: a transplant experiment in two Italian hotspots” submitted to *Marine Pollution Bulletin* in November 2021.

### **4.1 Background**

In port areas, NIS colonize man-made infrastructures and compete with native species, becoming an important component of biofouling communities (Connell & Glasby 1999, Canning-Clode et al. 2011, Campbell & Hewitt 2011, Brine et al. 2013, Ulman et al. 2019b, Culver et al. 2021). As a general rule, the success of NIS introduction, settlement, spread or proliferation in new environments is driven by both abiotic and biotic factors, which can be gathered into three main categories: physical-chemical factors (Piola & Johnston 2008, Piola et al. 2009, Canning-Clode et al. 2011); propagule pressure (Simberloff 2009, Simkanin et al. 2017); and ecological interactions within the biological communities present in the introduction area (Colautti et al. 2006, Occhipinti-Ambrogi 2007, Johnston et al. 2017).

Environmental factors in ports – e.g., temperature, nutrients, irradiance – can provide several ecological niches, suitable for NIS settlement (Ulman et al. 2019a). Moreover, habitat structure, like the type of substrate, can influence NIS colonization success. The design of the main artificial coastal structures (like walls, breakwaters and jetties) usually features steep slopes and low structural complexity; which can favour NIS colonization, and homogenization of biota at local, regional and global scales (Airoldi et al. 2015, Perkol-Finkel et al. 2018). The second factor, propagule pressure of NIS, encompasses the sizes, numbers, and temporal-spatial patterns of the propagules arriving in a recipient area (Simberloff 2009). This pressure can vary depending both on the type of vector and on the rate of propagule mortality during transport stage (Balestri et al. 2018, Briski et al. 2018). Moreover, it has been shown that propagule composition, including genetic variation and epigenetic potential, can play a role as relevant as the propagule pressure itself in determining invasion success (Briski et al. 2018). Lastly, intra- and interspecific interactions in the communities have been suggested as playing a pivotal role in determining the success or failure of NIS growth (Stachowicz et al. 2002), although the underlying ecological mechanisms are still unclear and have to be disentangled.

#### **4.1.1 Biotic resistance hypothesis and case studies**

Specifically, the theory of biotic resistance (Elton, C.S. 1958) predicts that species-rich communities (i.e. healthy native communities) should be less susceptible to invasion, due to a more complete use of resources (e.g. space and nutrients) by native species, which make them more resistant against new colonizers (Robinson et al. 1995, Stachowicz et al. 1999). Since the formulation of this hypothesis, further studies showed that native and NIS can actually establish not only negative relationships like the biotic resistance (Stachowicz et al. 2002, Balestri et al. 2018, Guilhem et al. 2020), but also positive relationships like facilitation (Levine & D'Antonio 1999, Bulleri et al. 2008, Rius & McQuaid 2009, Zwerschke et al. 2018). These divergent results lead to the invasion paradox: do native communities facilitate or hamper NIS introduction (Fridley et al. 2007)? The role of Marine Protected Areas (MPAs) in the protection from bioinvasions illustrate well the nature of the invasion paradox. MPAs are an essential tool for marine conservation as they are usually assigned to provide resilience of native communities to anthropogenic disturbances, including NIS introduction, while contributing with positive effects on other ecosystem services (Blanco et al. 2020). In MPAs in particular, since the native communities are presumably preserved and so introduced NIS do not have their own predators or parasites, NIS can even have better performance than their native counterparts (i.e., the so called “enemy-release” hypothesis; Liu & Stiling 2006, Burfeind et al. 2013).

Examples of putative NIS facilitation in MPAs were presented in Burfeind et al. (2013), that compared NIS assemblages inside and outside Atlantic and Pacific MPAs, and in no case a “resistance” to NIS was highlighted; on the contrary, NIS showed equal or higher performance inside protected areas than outside. On the contrary, Ardura et al. (2016) studied the presence of non-indigenous molluscs in two Pacific MPAs, underlining a negative correlation between NIS and dimension/level of protection of MPAs, assessing that protection should provide a valuable barrier against NIS introduction. Giakoumi & Pey (2017), starting from Burfeind et al. (2013), reviewed further case studies, including examples from the Mediterranean Sea, and showed that the density of target NIS was greater outside than inside MPAs, supporting the mechanism of biotic resistance of protected areas, in contrast with the results of the original study. In the Eastern Mediterranean, several NIS from the Suez Canal, including algae, invertebrates and fishes, were reported in MPAs and in some cases these NIS were dominant in the area (Galil et al. 2017). Regarding non-indigenous macroalgae in MPAs, two recent studies came to opposite conclusions. In Saõ Miguel Island (Azores), Cacabelos et al. (2019) highlighted a poor performance of the local MPA on limiting the distribution of two non-indigenous macroalgae. On the other hand, Blanco et al. (2020) found that two MPAs in the Iberian Peninsula are likely reducing the biomass of six invasive macroalgae, although not to prevent their establishment. The presence of non-indigenous fish species in several

South and Eastern Mediterranean MPAs in comparison with unprotected areas was investigated by Giakoumi et al. (2018), assessing a negative relationship between the richness of native species and NIS, as well as between NIS biomass and the distance from the Suez Canal; NIS biomass resulted also higher inside MPAs than in adjacent unprotected areas. Furthermore, future climatic conditions seem to reveal an increasing suitability of Mediterranean MPAs for several non-indigenous fish species (D'Amen & Azzurro 2019). Furthermore, it has been observed how the recreational marine traffic is considered an important vector of fouling NIS introduction in protected sites, like MPAs or regional parks (Iacarella et al. 2020a b, Parretti et al. 2020), due to the limitation of other anthropogenic activities.

In any case, a manipulative approach is needed to test hypothesis on the effective resistance of native communities to NIS colonization. When considering marine fouling communities, experimental studies have been often used to test ecological processes involved in the invasion paradox. More in general, these assemblages are very useful to verify theoretical ecological processes.

In some cases, the role of competition was highlighted as one of the drivers for the biotic resistance (Stachowicz et al. 1999, 2002, Gestoso et al. 2017), which appears to be significant only when resources are limited and pioneer species are scarce (Stachowicz & Byrnes 2006). On the other hand, the role of facilitation in shaping fouling communities is still poorly studied, although it was recognized as a key relationship in marine communities of other ecosystems, like in salt marshes, mangroves and coral reefs (Stachowicz 2001, Bruno et al. 2003).

More in detail, Stachowicz et al. (1999) found that an increase of native species richness on experimental tiles intensified the resistance to NIS, probably due to the higher efficiency of these native species-rich communities in the use of the space and the other limiting resources of the experimental system. Kimbro et al. (2013) conducted a meta-analysis on 42 studies on biotic resistance in marine environments, and their findings suggest that marine biotic resistance could be dependent on geographic latitude, the habitat and the trophic level of the invader species. Finally, Gestoso et al. (2017) did a transplantation experiment of fouling communities from an MPA to a touristic marina (richer in NIS), testing the settlement capability of NIS on PVC panels colonized by a MPA fouling community in Madeira Island. They found that transplanted communities from MPA showed lower NIS cover, thus suggesting a sort of biotic resistance to NIS.

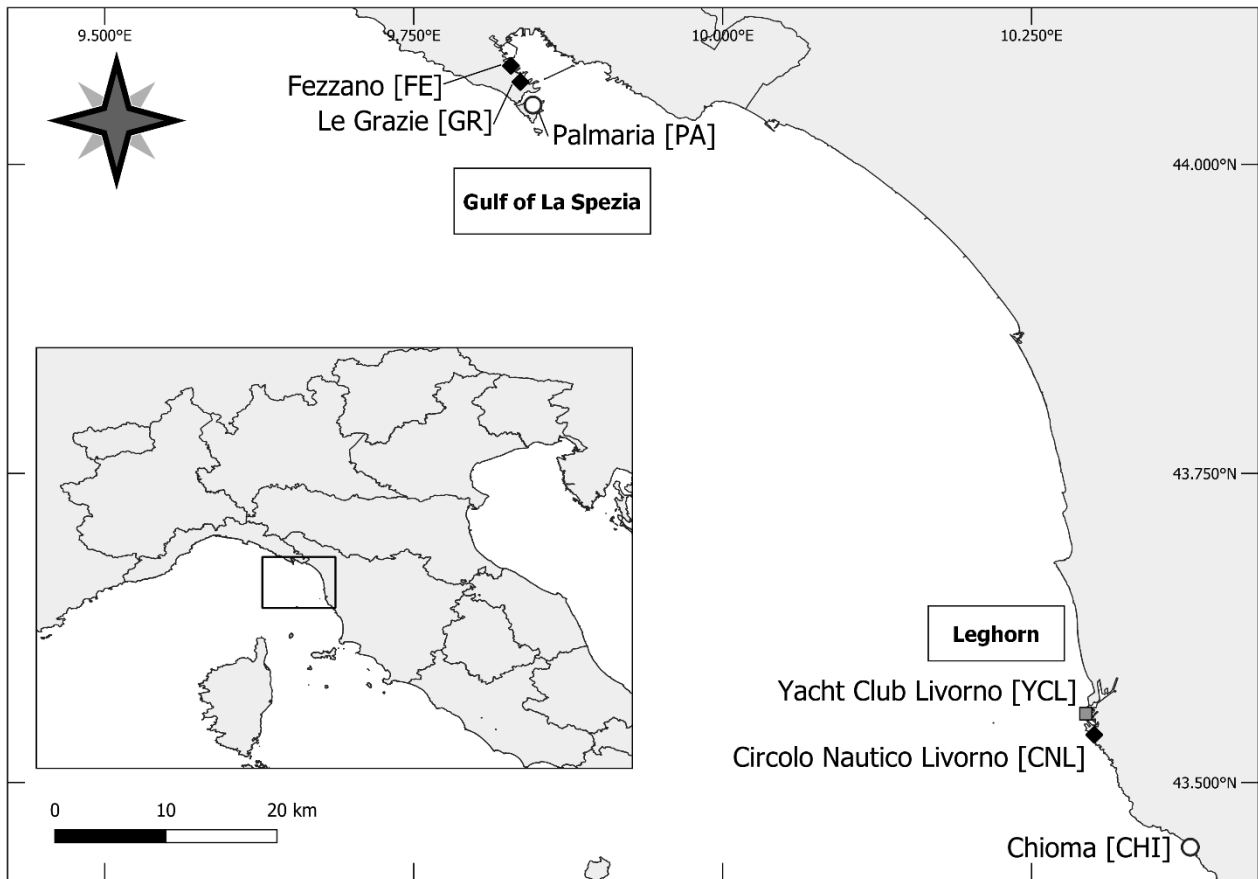
## 4.2 Aim of the study

Similarly to the study of Gestoso et al. (2017), the present work focused on a transplantation experiment of marine fouling communities in two sites belonging to the Ligurian Sea and to the Tyrrhenian Sea (Western Mediterranean, Italy) respectively. Fouling communities growing on PVC panels were moved from low impact sites (“natural” sites: a regional park near Cinque Terre, Ligurian Sea; and a rocky shore environment south to Leghorn, Tyrrhenian Sea) to high impact sites (“artificial” marinas known to host NIS; Tempesti et al. 2020, Tamburini et al. 2021), with the aim to assess the invasion success and the resistance to NIS of communities rich in native species. A difference in the structure of fouling communities from “natural” and “artificial” sites is expected, and the transplant experiments could provide information on the biotic resistance hypothesis. This in turn could result in useful information in order to select better management measures against NIS introduction in protected areas, as well as in ports.

## 4.3 Materials and methods

The hypothesis of higher biotic resistance of “natural” assemblages to NIS settlement was tested by a double transplant experiment, moving fouling communities grown on squared PVC panels (14 x 14 cm) from “natural” sites to marinas, affected by higher levels of NIS pressure, in two different study areas. The experiments were carried in the two areas in different years (2019 and 2020) during the summer season, in order to maximize the larval recruitment (Freestone et al. 2011, Marraffini et al. 2017).

Two study areas in Italy were chosen to carry out this experiment, the Gulf of La Spezia and the coastal area near Leghorn (Figure 4.1). In the first area the experiment was carried out in 2019, while in 2020 it was performed with the same method in Leghorn. The Gulf of La Spezia is one of the largest bays of the Ligurian Sea, about 5 km wide 144 and 10 km long, and is separated in two parts by an artificial offshore dam about 2 km long (Gasparini et al. 2009). It is characterized by both the presence of several anthropogenic activities, including a commercial harbor and several marinas with high NIS pressure (Tamburini et al. 2021), as well as “natural” areas, like the Palmaria Island (Ragazzola et al. 2017, 2021, Marchini et al. 2019), an UNESCO World Heritage site (<https://whc.unesco.org/en/list/826/>; accessed January 2022).

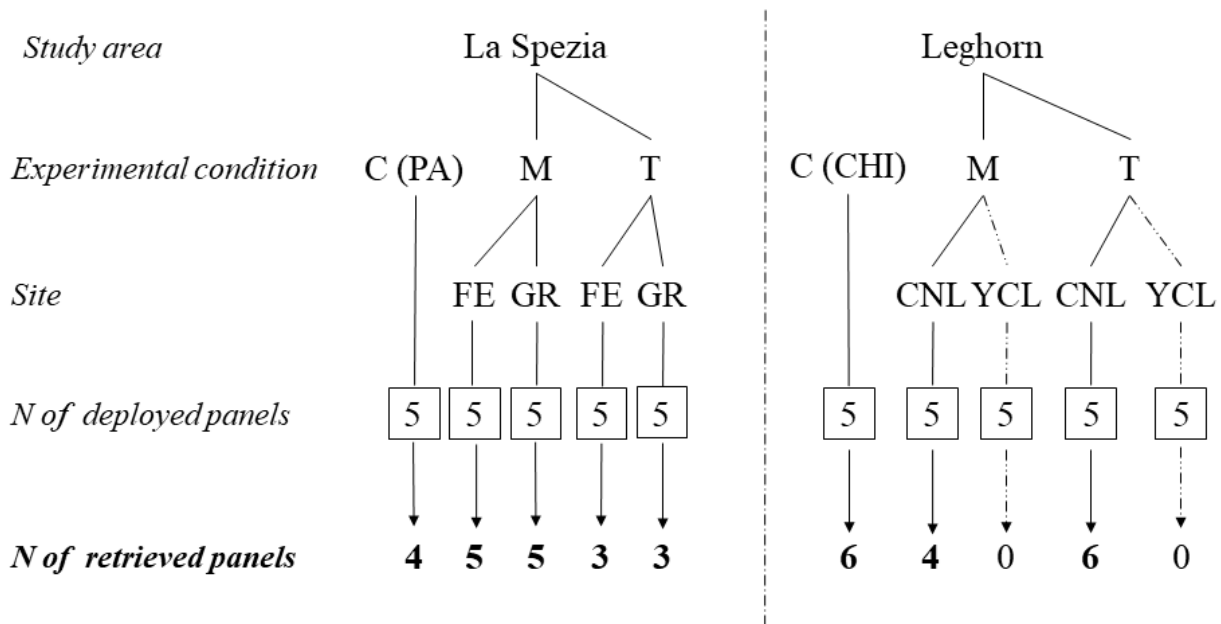


**Figure 4.1** Map of experimental sites. Black diamonds indicate the marina sites; white circles indicate the “natural” sites, used as donor sites; grey square indicate the marina site (namely “Yacht Club Livorno”) where the experiment was initially arranged but no panels were found for the transplantation.

For this study, two “natural” sites as donor sites in each study area were selected, the Palmaria Island (PA) in the Gulf of La Spezia and the locality of Chioma (CHI), an inlet in the province of Leghorn about 5 km from Calafuria, a rocky shore site with a good environmental status (Benedetti-Cecchi et al. 2001, Santangelo et al. 2012). A total of four sites with high NIS pressure were chosen as receiving sites, namely two marinas in the Gulf of La Spezia, Fezzano (FE) and Le Grazie (GR), and two marinas in Leghorn, “Circolo Nautico Livorno” (CNL) and “Yacht Club Livorno” (YCL), close to the commercial port of Leghorn (Figure 4.1, Table 4.1).

**Table 4.1 Geographical coordinates of sites.**

Site name	Site code	Latitude (° N)	Longitude (° E)
Palmaria	PA	44.047949	9.846488
Fezzano	FE	44.079643	9.828675
Le Grazie	GR	44.066539	9.836323
Chioma	CHI	43.447586	10.378522
Circolo Nautico Livorno	CNL	43.538444	10.300828
Yacht Club Livorno	YCL	43.548227	10.297766



**Figure 4.2 Design of the transplantation experiments in the two study areas. The arrows indicate the number of retrieved panels in each site and experimental condition. At the start of the experiment, 15 panels were deployed in both control sites (PA and CHI); after three months, 10 panels were transplanted from PA to FE (5) and GR (5) Due to the loss of all panels in YCL, the experimental design in the Leghorn area was modified: six panels (instead of five) were transplanted in CNL and six panels (instead of five) was maintained in CHI, as controls. In Leghorn study area, the only factor “experimental condition” was therefore considered during data analyses. C: control panels from donor sites; M: marina panels; T: transplanted panels; PA: Palmaria Island; FE: Fezzano marina; GR: Le Grazie marina; CHI: Chioma; CNL: marina “Circolo Nautico Livorno”; YCL: marina “Yacht Club Livorno”.**

The experimental units were prepared as exposed in paragraphs 2.3 and 3.3. The “natural” sites were provided with a small stretch of dock close to the rocky shores, where the experimental units have been suspended: in Palmaria the small dock is mainly dedicated to the ferries travelling within the Gulf of La Spezia, while in Chioma is reserved to small domestic recreational vessels (about 30 berths).

The panels were immersed for a total of five months: in 2019 from the end of April to the beginning of October, while in 2020 from the middle of May to the middle of October. The different immersion period between the two experimental years was due to logistical constraints following mobility restrictions related to the health emergency of Coronavirus disease (COVID-19).

In April 2019, 15 panels were deployed in PA, five in FE and five in GR. After three months (July 2019), only 10 panels in PA (probably due to vandalism or coastal storms) were found, which were collected, photographed, and rapidly analysed for species characterization, then: three panels were moved to GR (Transplanted panels condition: T), three panels to FE (T) and four panels were left in PA as control (Control condition: C). At the same time, the five panels in FE and the five in GR were photographed, rapidly analysed, and then left submerged as control in the marina sites (Marina condition: M; Figure 2). All panels (20) were then retrieved in October 2019. In 2020, the experiment was replicated in the Leghorn area. In May 15 panels were deployed in CHI, five panels in YCL and five in CNL. In August all the panels in YCL were lost, one panel in CNL and three panels in Chioma, so the experimental design had to be modified: YCL was excluded from the experiment, and we transplanted six panels from CHI to CNL (T). In addition, six panels were left in CHI (C) and four in CNL (M) as control panels. In October 2020 all remained panels (16) were retrieved (Figure 4.2).

After the retrieval, all panels were firstly preserved in ethanol 70% and then observed under dissecting microscopes for taxonomic identification of sessile invertebrates. The percent cover of the whole fouling assemblage was assessed by digital point count method (n=50 points) with photoQuad software (Trygonis & Sini 2012).

First of all, dataset from donor sites (C condition) were analysed separately, in order to verify the presumed low NIS pressure in the chosen “natural” sites. Then, species richness (S), NIS/native species ratio (Olenin et al. 2010), Shannon index ( $H'$ , based on  $\ln$ ) and percent cover of each taxon were calculated for each panel, considering only the sessile macroinvertebrates. A biogeographic status (Chapman & Carlton 1991) was assigned to each taxon, namely NIS, native species (including cryptogenic species - i.e. species that cannot be considered either native or introduced; see Carlton 1996) or unresolved taxa (due to insufficient taxonomic resolution e.g. damaged specimens or juveniles). Cryptogenic species were included in the group of native species following a conservative approach. Furthermore, the fact that several taxa were identified only to genus (or higher) taxonomic level (Supplementary table 2) did not allow us to assess with certainty their biogeographic status (Katsanevakis & Moustakas 2018). Nevertheless, in both study areas the cover of cryptogenic species in C, M and T conditions was also assessed for a comprehensive description of the dataset.



The datasets from the two study areas were analysed separately, due to the different experimental design applied and the different environmental conditions observed (Figure 4.2). For La Spezia dataset, differences in mean values of S, NIS/native species ratio, H', and percent cover of native species and NIS were assessed by two-way ANOVA tests (type III; fixed factor: "condition" with two levels: M, T; random factor: "site" with two levels: FE, GR; unequal sample size; Figure 4.2). For Leghorn dataset, differences in the same variables of La Spezia were assessed by Welch's t-tests (fixed factor "experimental condition" with two levels: M, T; Figure 4.2). In all the cases of non-homogeneity of the variances, data were properly transformed, and a more conservative critical value ( $\alpha < 0.01$ ) was chosen if the data transformation was not successful.

Non-metric multi-dimensional scaling (nmMDS) among centroids of samples (log transformed; resemblance matrix obtained using Bray-Curtis similarity) was used to visualize the pattern of fouling communities of C, M and T panels in both study areas at the end of the experiment.

PERMDISP test and two-way crossed PERMANOVA (Anderson et al. 2008) were carried out to test differences within and between factors "condition" (fixed, two levels: M, T) and "site" (random, two levels: FE, GR) in La Spezia dataset, while in Leghorn dataset PERMDISP and one-way PERMANOVA were employed to test differences within factor "condition". Moreover, SIMPER analyses showed the contribution of NIS to the dissimilarities between experimental conditions in both study areas. All the analyses were carried out with the software Microsoft Excel, R (R Core Team 2017), QGIS (QGIS Development Team 2020) and PRIMER 6 with PERMANOVA+ add-on package (Clarke & Gorley 2006, Anderson et al. 2008).

## 4.4 Results

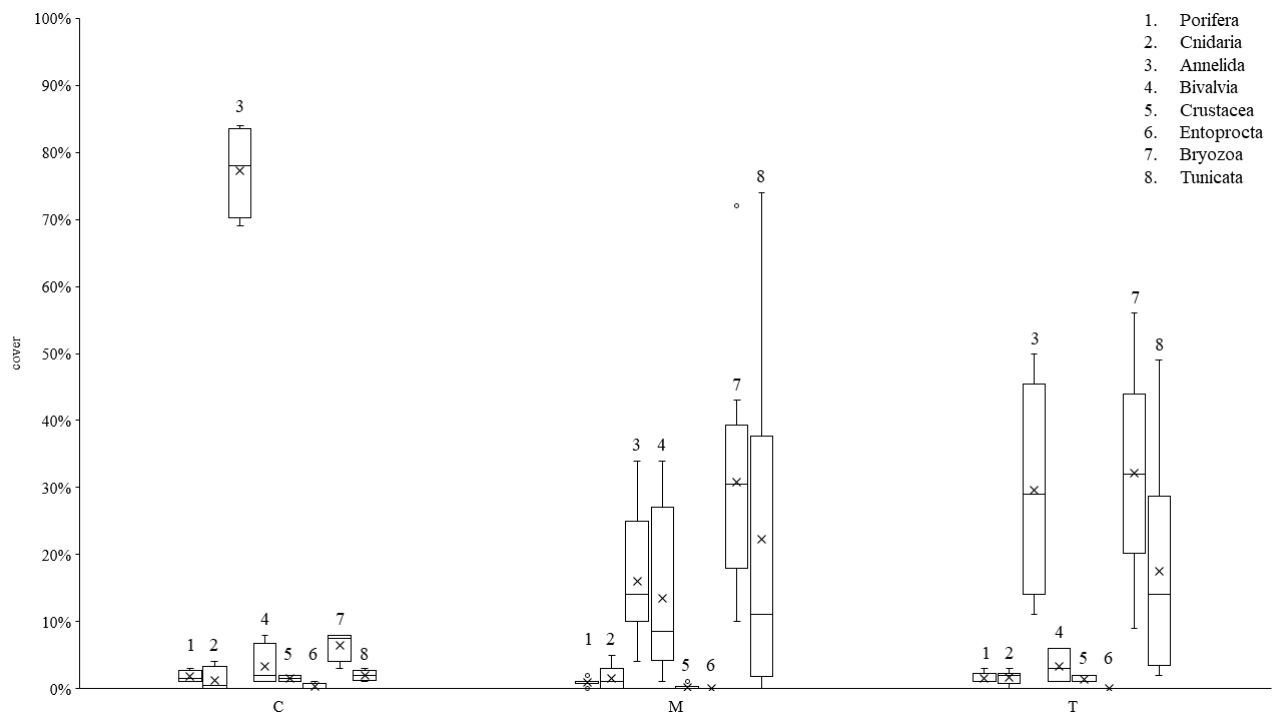
### *Gulf of La Spezia (2019)*

At the end of this experiment in the Gulf of La Spezia, five months from the first deployment of panels, an overall of 58 taxa of sessile macrozoobenthos belonging to eight taxonomic groups were found on PVC panels, regardless experimental condition (Supplementary table 2). As general description of the sampled assemblages, nine taxa were identified as NIS (Table 4.2), 43 as native (including cryptogenic species) and six taxa were considered as unresolved. The non-indigenous bivalve *Arcuatula senhousia* (Benson, 1842) and the non-indigenous bryozoan *Amathia verticillata* (delle Chiaje, 1822) were found only after three months of the experiment (Table 4.2). The most represented group was Bryozoa (19 taxa), followed by Annelida (12), Cnidaria, Bivalvia and Tunicata (7).

**Table 4.2 List of NIS found in La Spezia study area in all experimental conditions and sites before transplant (three months: t3) and after transplant (five months: t5). NIS with “\*” indicate species found in panels only before transplant (t3). For more details on *Branchiomma* sp. identification, see chapter 3. PA: Palmaria Island; FE: Fezzano marina; GR: Le Grazie marina. C: control panels, which remained for all months in PA; M: marina panels, which remained for all months in FE or GR; T: transplanted panels, which were firstly located in PA (t3) and then transplanted from PA to FE or to GR (t5).**

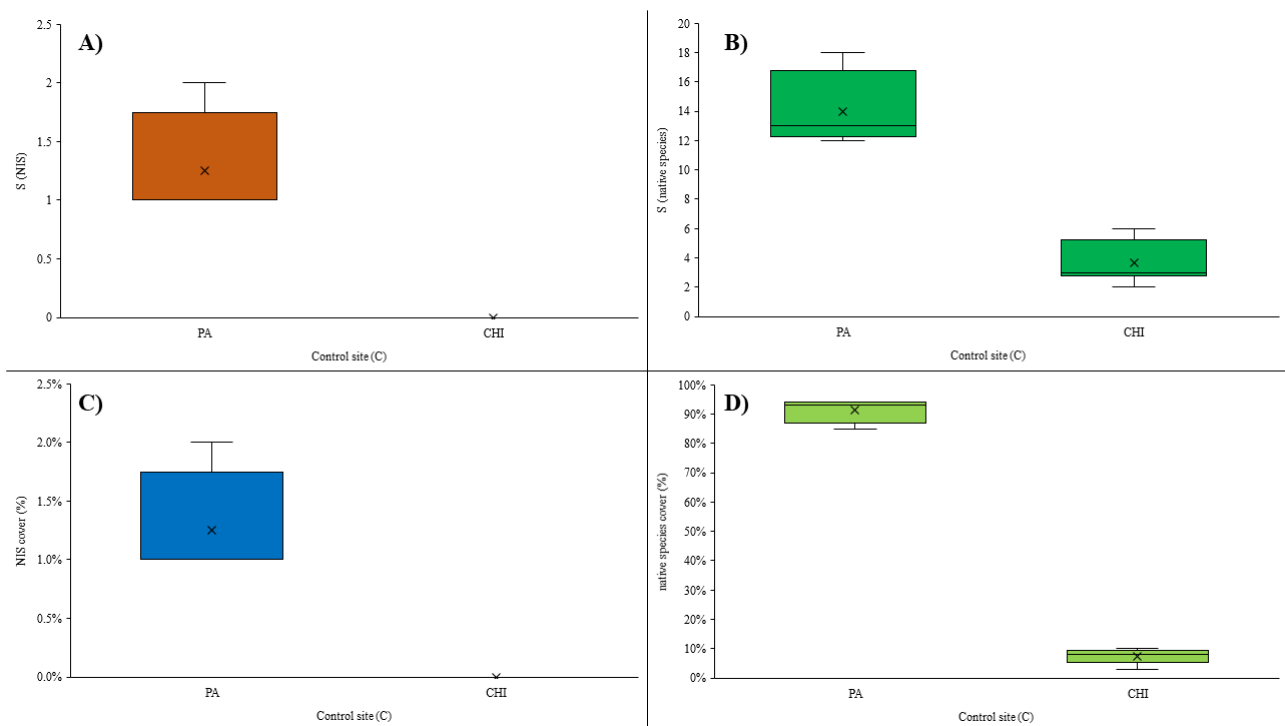
		PA		FE				GR			
		C		M		T		M		T	
		t3	t5	t3	t5	t3	t5	t3	t5	t3	t5
<b>Porifera</b>	<i>Paraleucilla magna</i> Klautau, Monteiro & Borojevic, 2004						•				
<b>Annelida</b>	<i>Branchiomma</i> sp.		•	•	•	•		•	•	•	
	<i>Branchiomma luctuosum</i> (Grube, 1870)			•	•			•			•
	<i>Hydroides dirampha</i> Mörch, 1863				•			•	•	•	
	<i>Hydroides elegans</i> (Haswell, 1883)				•			•	•	•	
<b>Bivalvia</b>	<i>Arcuatula senhousia</i> (Benson, 1842)	•		•	•	•	•	•	•	•	
<b>Bryozoa</b>	<i>Amathia verticillata</i> (delle Chiaje, 1822)*			•							
	<i>Celleporaria brunnea</i> (Hincks, 1884)			•	•			•	•	•	•
	<i>Tricellaria inopinata</i> d'Hondt & Occhipinti Ambrogi, 1985		•		•		•				
	<i>Watersipora arcuata</i> Banta, 1969	•							•		
<b>Tunicata</b>	<i>Styela plicata</i> (Lesueur, 1823)		•	•	•		•	•	•		•

In the study area, at the end of the experiment, Annelida showed the highest mean percentage cover value ( $\pm$  SD) in C ( $77\pm 7\%$ ), Bryozoa in M ( $32\pm 17\%$ ) and in T ( $32\pm 16\%$ ) (Figure 4.3).



**Figure 4.3 Boxplot of taxonomic groups found on panels in the experimental conditions at the end of the experiment (five months) in the Gulf of La Spezia. C: control panels; M: marina panels; T: transplanted panels. X in the boxplots indicate mean values.**

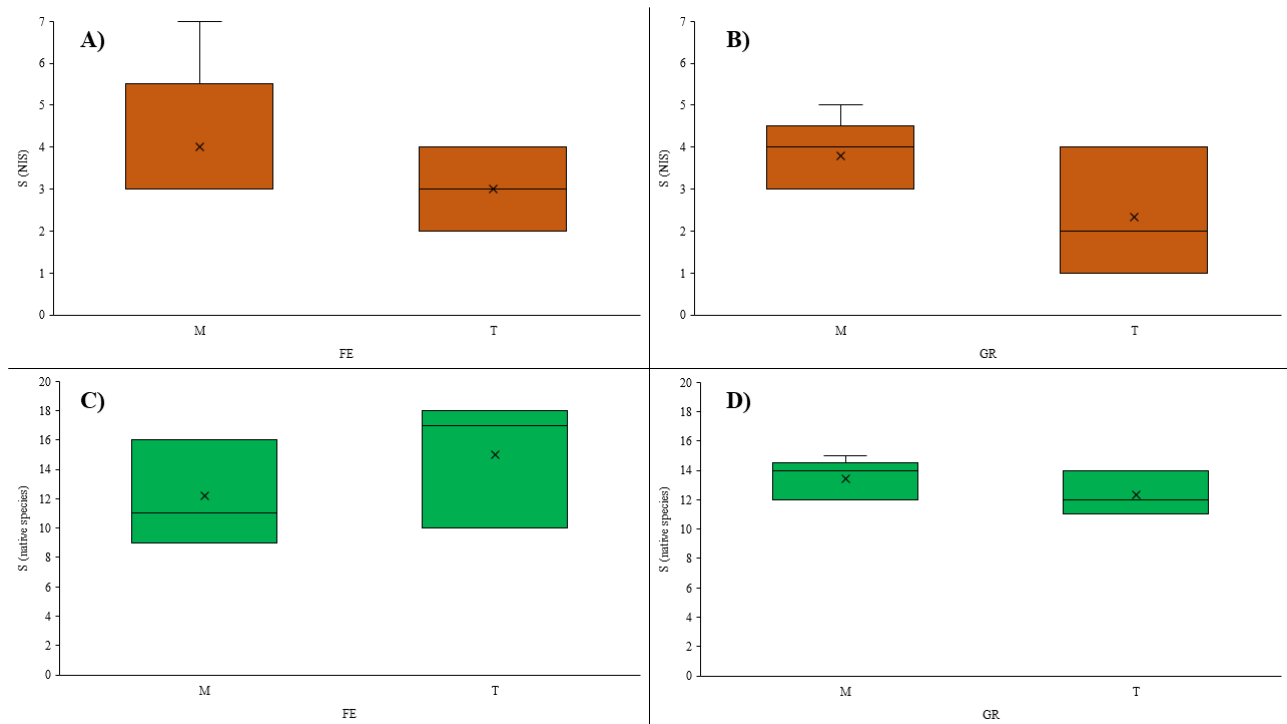
As expected, NIS pressure in the Palmaria Island (control site, C) was negligible (Table 4.2). At the end of the experiment (five months), the mean NIS number was  $1 \pm 1$  (Figure 4.4A) and NIS cover was  $1 \pm 1\%$  (Figure 4.4C); while the number ( $14 \pm 3$ ) and cover ( $91 \pm 4\%$ ) of native species were high (Figure 4.4B and D).



**Figure 4.4** Boxplots of number of NIS (A), number of native species (B) and cover of NIS (C) and native species (D) of the two control sites of this study. PA: Palmaria Island; CHI: Chioma. X in the boxplots indicate mean values.

Regarding NIS number in the marinas of La Spezia study area, the highest mean values were found in both M conditions, FE ( $4.0 \pm 1.7$ ) and GR ( $3.8 \pm 0.8$ ), while in T the values were lower, respectively  $3.0 \pm 1.0$  in FE and  $2.3 \pm 1.5$  in GR (Table 4.3, Figure 4.5A-B). Two-way crossed ANOVA test showed no significant difference among values both in condition ( $F = 1.0481$ ,  $p = 0.3261$ ) and in site ( $F = 0.0559$ ,  $p = 0.8171$ ), and no interaction between condition and site was highlighted ( $F = 0.1141$ ,  $p = 0.7413$ ; Table 4.4).

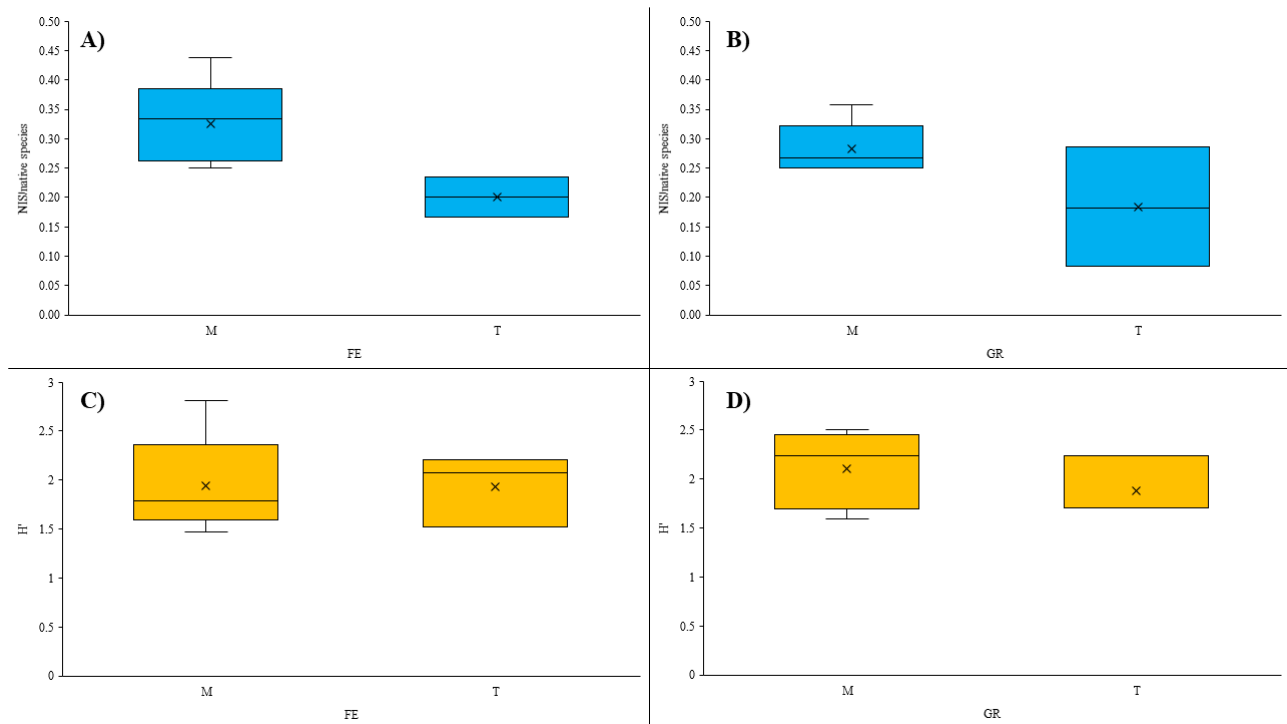
The mean number of native species (cryptogenic species included) was the highest in T panels in FE ( $15.0 \pm 4.4$ ) and in M in GR ( $13.4 \pm 1.3$ ), followed by T in GR ( $12.3 \pm 1.5$ ) and M in FE ( $12.2 \pm 3.6$ ; Table 4.3, Figure 4.5C-D), but ANOVA results highlighted no differences between condition ( $F = 1.7523$ ,  $p = 0.2102$ ), site ( $F = 0.4291$ ,  $p = 0.5248$ ), and factors interaction ( $F = 1.6709$ ,  $p = 0.2205$ ; Table 4.4).



**Figure 4.5** Boxplots of: NIS number in M and T panels after five months in FE (A) and in GR (B); native species number in M and T panels after five months in FE (C) and in GR (D). M: marina panels; T: transplanted panels; FE: Fezzano; GR: Le Grazie. X in the boxplots indicate mean values.

The highest mean values of NIS/native species ratio were found in M both in FE ( $0.33 \pm 0.07$ ) and in GR ( $0.28 \pm 0.04$ ), while the values resulted lower in T, respectively  $0.20 \pm 0.03$  in FE and  $0.18 \pm 0.10$  in GR (Table 4.3, Figure 4.6A-B). ANOVA test showed significant difference in condition ( $F = 6.7396$ ,  $p = 0.02339$ ), but not in site ( $F = 1.0918$ ,  $p = 0.31668$ ), and no interaction was highlighted ( $F = 0.1515$ ,  $p = 0.70396$ ; Table 4.4).

Mean  $H'$  in FE was  $1.94 \pm 0.51$  in M and  $1.93 \pm 0.37$  in T, while in GR it was  $2.11 \pm 0.40$  in M and  $1.88 \pm 0.31$  in T (Table 4.3, Figure 4.6C-D). ANOVA highlighted no differences between condition ( $F = 0.0002$ ,  $p = 0.9887$ ), site ( $F = 0.4030$ ,  $p = 0.5374$ ), and factors interaction ( $F = 0.2505$ ,  $p = 0.6258$ ; Table 4.4).

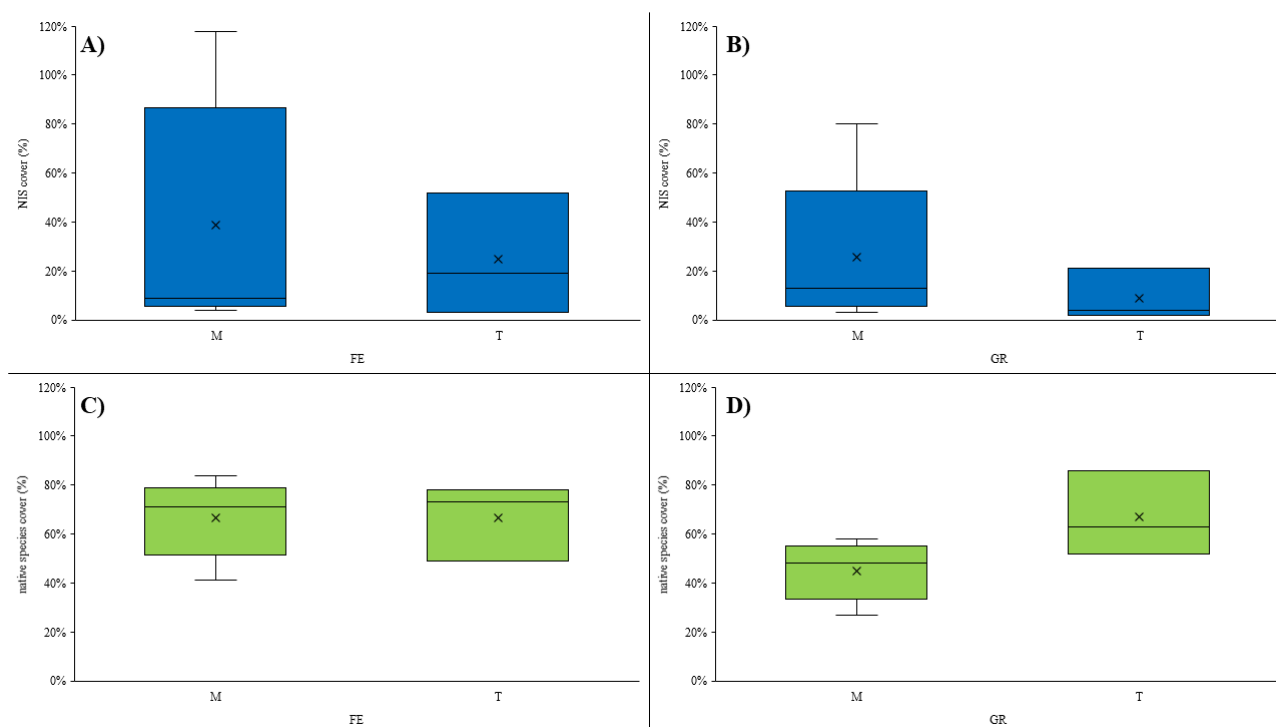


**Figure 4.6** Boxplots of: NIS/native species ratio in M and T panels after five months in FE (A) and in GR (B); Shannon index (H') in M and T panels after five months in FE (C) and in GR (D). M: marina panels; T: transplanted panels; FE: Fezzano; GR: Le Grazie. X in the boxplots indicate mean values.

Regarding NIS cover (%), the highest mean values were found in M both in FE ( $39 \pm 49\%$ ) and in GR ( $26 \pm 31\%$ ), while in T the values were lower, respectively  $25 \pm 25\%$  in FE and  $9 \pm 10\%$  in GR (Table 4.3, Figure 4.7A-B). Two-way crossed ANOVA test showed no significant difference among values both in condition ( $F = 0.2902$ ,  $p = 0.59993$ ) and in site ( $F = 0.3266$ ,  $p = 0.57823$ ), and no interaction between condition and site was highlighted ( $F = 0.0061$ ,  $p = 0.93882$ ; Table 4.4).

Mean native species cover (%) in FE was  $66 \pm 16\%$  in M and  $67 \pm 16\%$  in T, while in GR it was  $45 \pm 12\%$  in M and  $67 \pm 17\%$  in T (Table 4.3, Figure 4.7C-D). ANOVA results showed no differences between condition ( $F = 0.0006$ ,  $p = 0.98102$ ) and factors interaction ( $F = 1.9595$ ,  $p = 0.18689$ ), but a significant difference between sites ( $F = 5.0662$ ,  $p = 0.04393$ ; Table 4.4).

Moreover, cryptogenic species found in this study area are reported in supplementary table 2. Their mean cover was  $2 \pm 1\%$  in C,  $8 \pm 6\%$  in M and  $4 \pm 4\%$  in T.



**Figure 4.7** Boxplots of: NIS cover (%) in M and T panels after five months in FE (A) and in GR (B); native species cover (%) in M and T panels after five months in FE (C) and in GR (D). M: marina panels; T: transplanted panels; FE: Fezzano; GR: Le Grazie. X in the boxplots indicate mean values.

**Table 4.3** Mean values of the investigated variables in M and T conditions in both sites in the Gulf of La Spezia. M: marina panels; T: transplanted panels; FE: Fezzano; GR: Le Grazie.

	FE		GR	
	M	T	M	T
<i>S</i> (NIS)	4.0±1.7	3.0±1.0	3.8±0.8	2.3±1.5
<i>S</i> (native species)	12.2±3.6	15.0±4.4	13.4±1.3	12.3±1.5
<i>NIS/native species</i>	0.33±0.07	0.20±0.03	0.28±0.04	0.18±0.10
<i>H'</i>	1.97±0.52	1.93±0.37	2.11±0.40	1.88±0.31
<i>NIS cover (%)</i>	28±29%	25±25%	26±31%	9±10%
<i>native species cover (%)</i>	61±22%	67±16%	45±12%	67±17%

**Table 4.4 Two-way crossed ANOVAs (type III; fixed factor: “condition” with two levels: M, T; random factor: “site” with two levels: FE, GR; unequal sample size) on the investigated variables in the Gulf of La Spezia.**

<i>S (NIS)</i>					<i>S (native species)</i>				
	Sum Sq	Df	F value	Pr(>F)		Sum Sq	Df	F value	Pr(>F)
<i>Condition</i>	1.875	1	1.0481	0.3261 ns	<i>Condition</i>	14.70	1	1.7523	0.2102 ns
<i>Site</i>	0.100	1	0.0559	0.8171 ns	<i>Site</i>	3.60	1	0.4291	0.5248 ns
<i>Co x Si</i>	0.204	1	0.1141	0.7413 ns	<i>Co x Si</i>	14.02	1	1.6709	0.2205 ns
<i>Residuals</i>	21.467	12			<i>Residuals</i>	100.67	12		
<i>NIS/native species</i>					<i>H'</i>				
	Sum Sq	Df	F value	Pr(>F)		Sum Sq	Df	F value	Pr(>F)
<i>Condition</i>	0.02917	1	6.7396	0.02339 *	<i>Condition</i>	0.0020	1	0.0111	0.9179 ns
<i>Site</i>	0.00472	1	1.0918	0.31668 ns	<i>Site</i>	0.0500	1	0.2797	0.6065 ns
<i>Co x Si</i>	0.00066	1	0.1515	0.70396 ns	<i>Co x Si</i>	0.0339	1	0.1896	0.6710 ns
<i>Residuals</i>	0.05193	12			<i>Residuals</i>	2.1443	12		
<i>NIS cover (%)</i>					<i>native species cover (%)</i>				
	Sum Sq	Df	F value	Pr(>F)		Sum Sq	Df	F value	Pr(>F)
<i>Condition</i>	0.00161	1	0.0222	0.88414 ns	<i>Condition</i>	0.0069	1	0.2275	0.6419 ns
<i>Site</i>	0.00081	1	0.0111	0.91774 ns	<i>Site</i>	0.06084	1	2.0058	0.1821 ns
<i>Co x Si</i>	0.01803	1	0.2476	0.62776 ns	<i>Co x Si</i>	0.0238	1	0.7847	0.3931 ns
<i>Residuals</i>	0.87367	12			<i>Residuals</i>	0.36399	12		

Referring to the multivariate analyses of the communities on panels at the end of the experiment, non-metric MDS (2D stress: 0.15) showed a separation between M and T panels, but not a clear separation between FE and GR sites (Figure 4.8A). Two-way PERMANOVA revealed a significant difference between conditions (Pseudo-F = 9.3039, unique permutations: 6, p(Monte Carlo) = 0.008), but not between sites (Pseudo-F = 0.75516, unique permutations: 9934, p(perm) = 0.623), and no interaction between factors was highlighted (Pseudo-F = 0.25052, unique permutations: 9932, p(perm) = 0.9534). PERMDISP test on factor condition excluded a dispersion effect of samples (F= 3.1291, p(perm) = 0.096).

SIMPER analysis revealed average similarity within each condition, average dissimilarity between conditions (Table 4.5) and taxa major contribution to these values (Supplementary tables 3-4). In general, there are more NIS in M than in T among the taxa most contributing to similarity (Supplementary table 3). More in detail, the taxa contributing most (>10%) to the differentiation between communities of M and T panels were the serpulid *Salmacina* sp., the bryozoan *Crisia* sp. and the non-indigenous tunicate *Styela plicata* (Lesueur, 1823), which contributed with 14.04%, 13.86% and 13.58%, respectively (Supplementary table 4).

**Table 4.5 Percentage average similarity within conditions and average dissimilarities among conditions of experimental fouling communities in the Gulf of La Spezia.**

Average Similarity		Average Dissimilarity	
<b>C</b>	76.84	<b>C &amp; M</b>	85.22
<b>M</b>	33.83	<b>C &amp; T</b>	61.01
<b>T</b>	37.57	<b>M &amp; T</b>	71.04

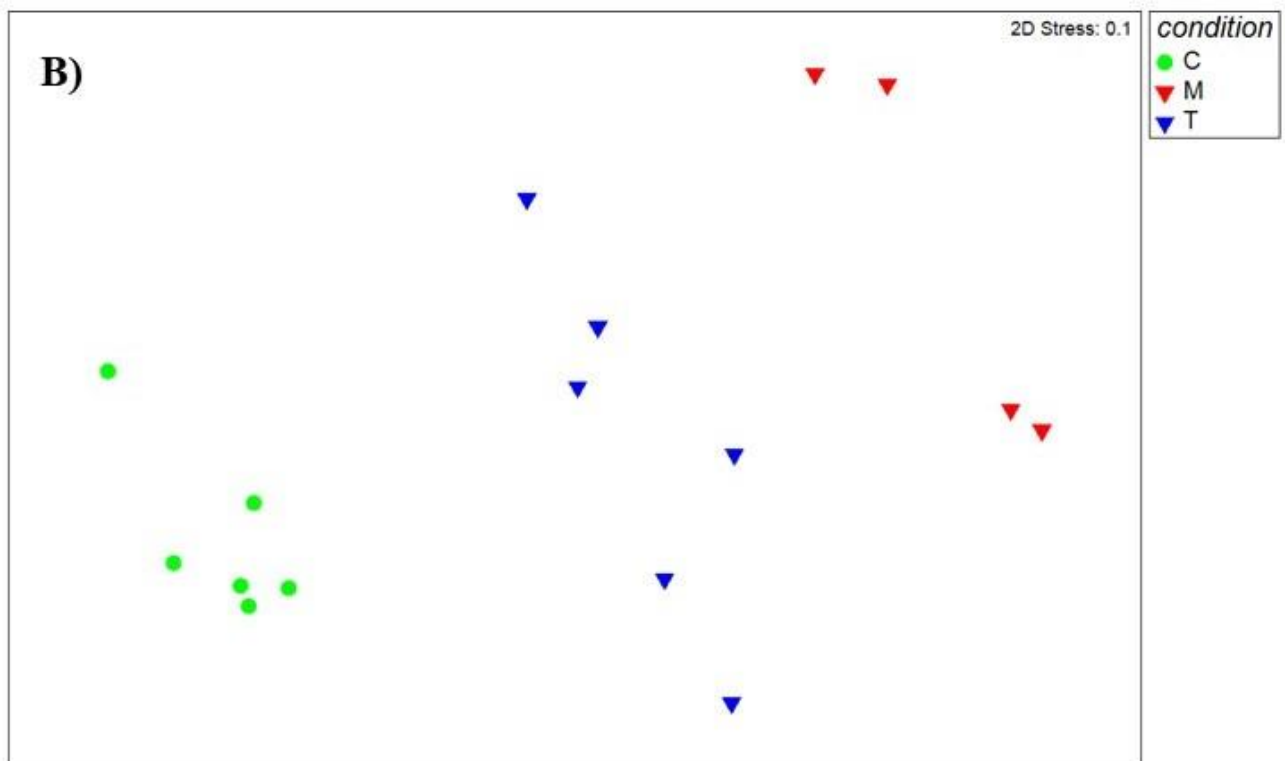
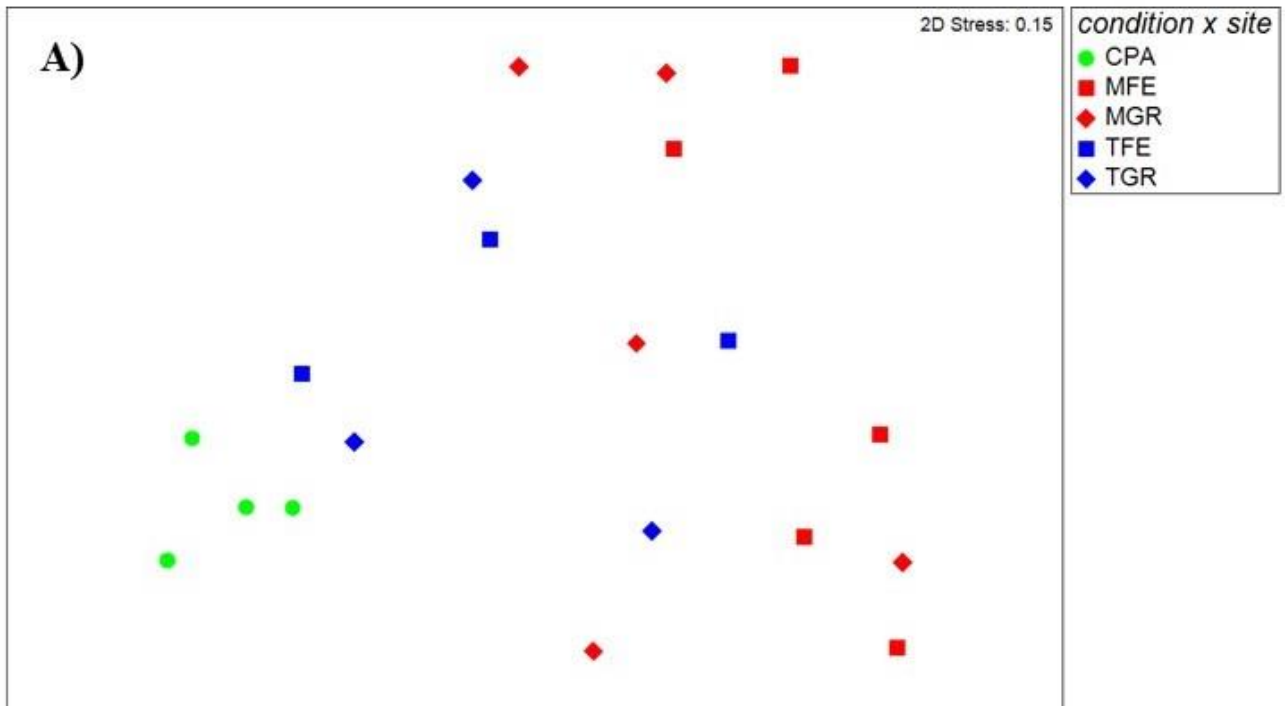


Figure 4.8 nmMDS on Bray Curtis similarity (log transformed data) among panels of C, M and T conditions at the end of the experiment (five months). A) Gulf of La Spezia (PA: Palmaria; FE: Fezzano; GR: Le Grazie); B) Leghorn area. C: control panels; M: marina panels; T: transplanted panels.



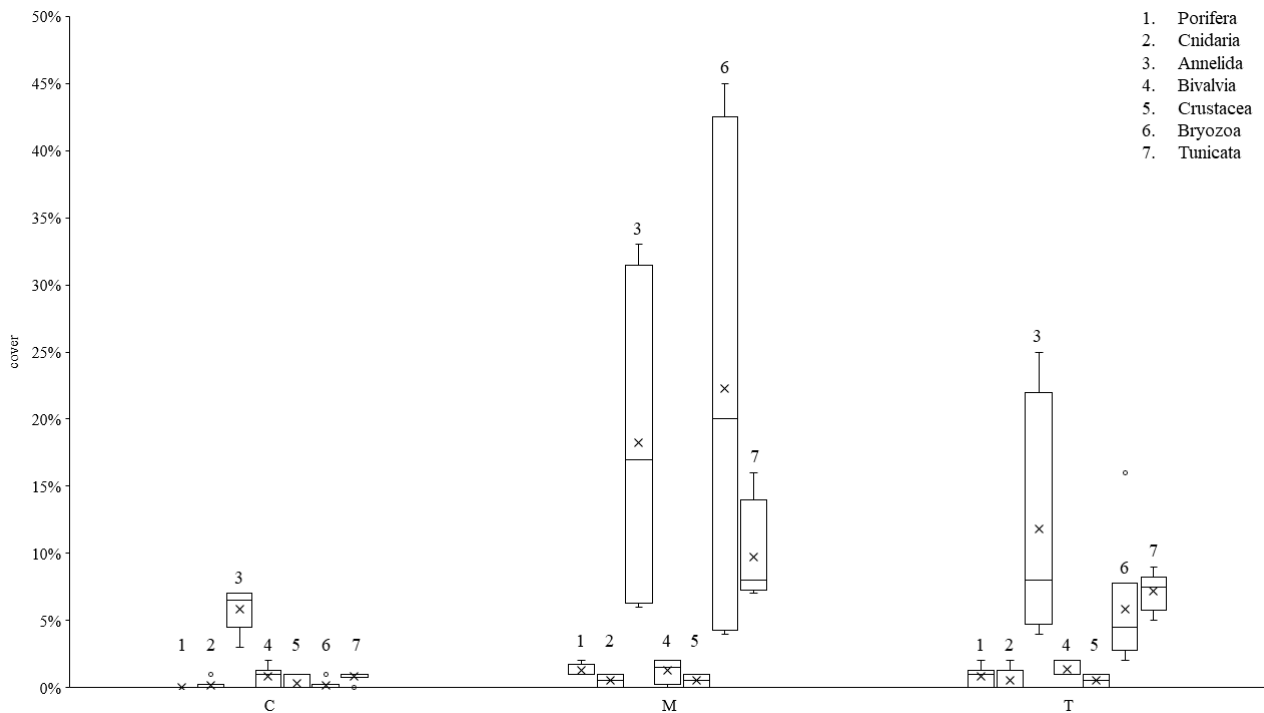
### *Leghorn area (2020)*

In the panels from Leghorn area, a total of 36 taxa from seven taxonomic groups of sessile macrozoobenthic species were found (Supplementary table 2). Ten taxa were identified as NIS (Table 4.6), 21 as native (including cryptogenic species) and five taxa were considered as unresolved. The most represented groups were Annelida (10 taxa), Bryozoa and Tunicata (8).

**Table 4.6 List of NIS found in Leghorn study area in all experimental conditions before transplant (three months: t3) and after transplant (five months: t5). Panels T (t3) were transplanted from CHI (Chioma) to the marina site (Circolo Nautico Livorno). For more details on *Branchiomma* sp. identification, see chapter 3. C: control panels; M: marina panels; T: transplanted panels.**

		C (CHI)		M		T	
		t3	t5	t3	t5	t3	t5
<b>Porifera</b>	<i>Paraleucilla magna</i> Klautau, Monteiro & Borojevic, 2004			•			
<b>Annelida</b>	<i>Branchiomma</i> sp.					•	
	<i>Ficopomatus enigmaticus</i> (Fauvel, 1923)			•			
	<i>Hydroides dirampha</i> Mörch, 1863			•			
	<i>Hydroides elegans</i> (Haswell, 1883)			•	•		•
<b>Bryozoa</b>	<i>Amathia verticillata</i> (delle Chiaje, 1822)			•	•		
	<i>Celleporaria brunnea</i> (Hincks, 1884)						•
	<i>Tricellaria inopinata</i> d'Hondt & Occhipinti Ambrogi, 1985						•
<b>Tunicata</b>	<i>Botrylloides</i> cf. <i>niger</i> Herdman, 1886			•	•		•
	<i>Styela plicata</i> (Lesueur, 1823)				•		

The cover (%) of taxonomic groups varied among experimental conditions in the study area at the end of the experiment. In particular, Annelida showed the highest mean value ( $\pm$  SD) in C ( $77\pm 7\%$ ), Bryozoa in M ( $32\pm 17\%$ ) and in T ( $32\pm 16\%$ ) (Figure 4.9).



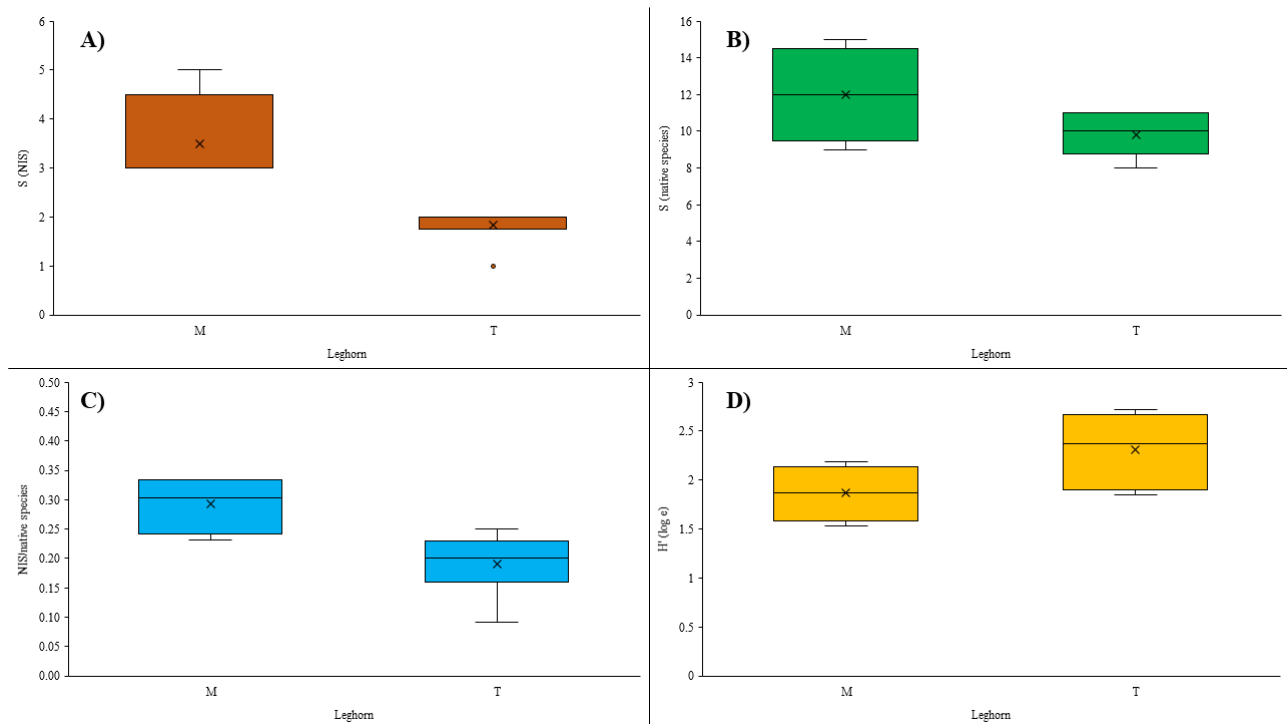
**Figure 4.9** Boxplot of taxonomic groups found on panels in the experimental conditions at the end of the experiment (five months) in the Leghorn area. C: control panels; M: marina panels; T: transplanted panels; X in the boxplots indicate mean values.

At the end of the experiment, in Chioma (control site, C) NIS resulted absent (Table 4.6, Figure 4A, 4C); while native species number and cover were low,  $4 \pm 2$  and  $7 \pm 3\%$  respectively (Figure 4.10B and D).

Regarding NIS richness (S) in the study area, the mean value in M condition was  $3.5 \pm 1.0$ , while in T condition it was  $1.8 \pm 0.4$  (Table 4.7, Figure 4.10A). A significant difference between M and T conditions was highlighted by Welch's *t*-test ( $t = 3.1623$ ,  $p = 0.03835$ ; Table 4.8).

The mean number of native species resulted  $12.0 \pm 2.6$  in M and  $9.8 \pm 1.2$  in T (Table 4.7, Figure 4.10B) and no difference was observed ( $t = 1.5742$ ,  $p = 0.1936$ ; Table 4.8). On the other hand, the mean NIS/native ratio was  $0.29 \pm 0.05$  in M,  $0.19 \pm 0.05$  in T (Table 4.7, Figure 4.10C) and a significant difference was highlighted ( $t = 3.0411$ ,  $p = 0.01893$ ; Table 4.8).

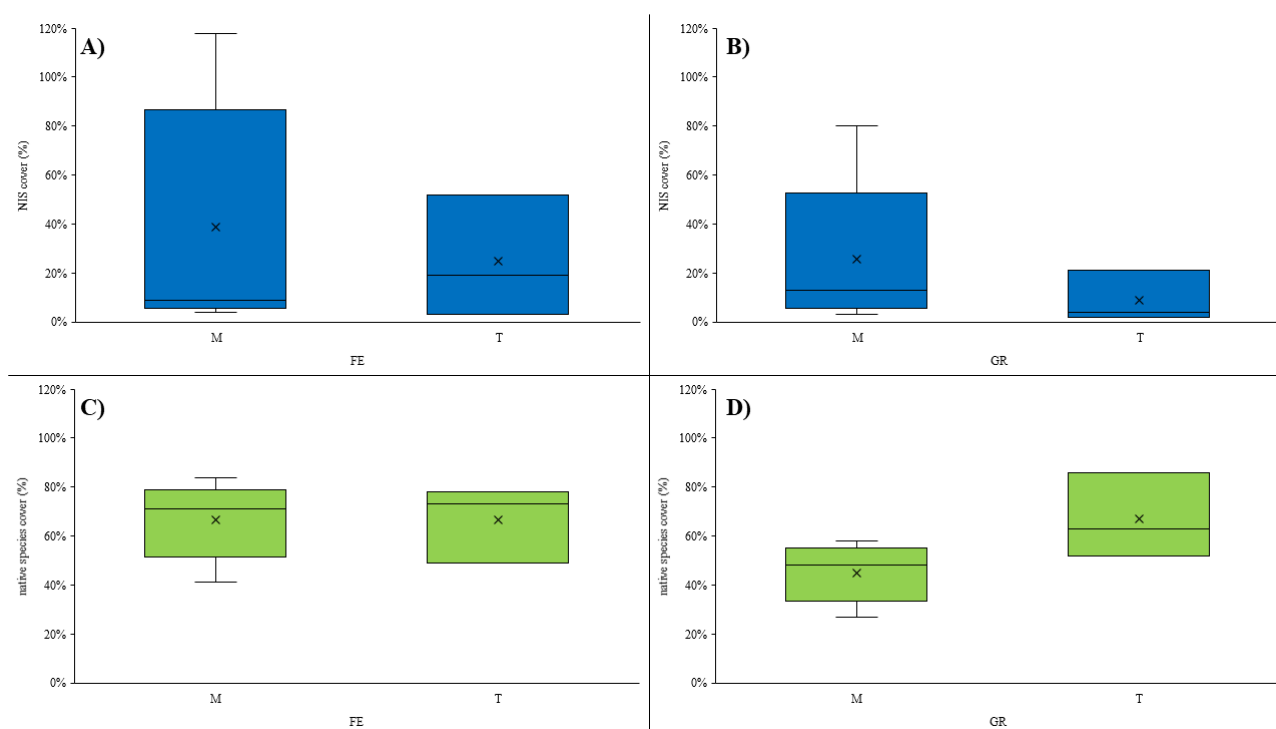
Mean  $H'$  in M was  $1.87 \pm 0.28$ , while in T was  $2.31 \pm 0.39$  (Table 4.7, Figure 10D), but no significant difference was observed ( $t = -2.0826$ ,  $p = 0.07149$ ; Table 4.8).



**Figure 4.10** Boxplots of: NIS (A) and native species (B) number, NIS/native species ratio (C) and Shannon index  $H'$  (D) in M and T panels after five months in the Leghorn study area. M: marina panels; T: transplanted panels. X in the boxplots indicate mean values.

Mean NIS cover (%) was the highest in M ( $6 \pm 4\%$ ) and the lowest in T ( $3 \pm 1\%$ ) (Table 4.7, Figure 4.11A), but no significant difference was highlighted ( $t = 1.6408$ ,  $p = 0.1974$ ; Table 4.8). On the other hand, mean native species cover (%) was  $43 \pm 9\%$  in M,  $21 \pm 9\%$  in T (Table 4.7, Figure 11B), and a significant difference was observed ( $t = 3.7621$ ,  $p = 0.007835$ ; Table 4.8).

Cryptogenic species found in this study area are reported in supplementary table 2. Mean cover of these species was  $0.3 \pm 0.5\%$  in C,  $22 \pm 20\%$  in M and  $7 \pm 7\%$  in T.



**Figure 4.11** Boxplots of NIS (A) and native species (B) cover (%) in M and T panels after five months in the Leghorn study area. M: marina panels; T: transplanted panels. X in the boxplots indicate mean values.

**Table 4.7** Mean values of the investigated variables in M and T conditions in the Leghorn area. M: marina panels; T: transplanted panels.

	M	T
<i>S (NIS)</i>	3.5±1.0	1.8±0.4
<i>S (native species)</i>	12.0±2.6	9.8±1.2
<i>NIS/native species</i>	0.29±0.05	0.19±0.05
<i>H'</i>	1.87±0.28	2.31±0.39
<i>NIS cover (%)</i>	6±4%	3±1%
<i>native species cover (%)</i>	43±9%	21±9%

**Table 4.8** Welch's t-tests (fixed factor "experimental condition" with two levels: M, T) on the investigated variables in the Leghorn area.

<i>S (NIS)</i>	t	df	p-value	<i>S (native species)</i>	t	df	p-value
<i>Condition</i>	3.1623	3.6765	0.03835 *	<i>Condition</i>	1.5742	3.8331	0.1936 ns
<i>NIS/native species</i>	t	df	p-value	<i>H'</i>	t	df	p-value
<i>Condition</i>	3.0411	6.9672	0.01893 *	<i>Condition</i>	-2.0826	7.8514	0.07149 ns
<i>NIS cover (%)</i>	t	df	p-value	<i>native species cover (%)</i>	t	df	p-value
<i>Condition</i>	1.6408	3.0668	0.19740 ns	<i>Condition</i>	3.7621	6.6125	0.007835 **

Non-metric MDS (2D stress: 0.11) showed a separation between M and T panels (Figure 4.8B) and one-way PERMANOVA confirmed this separation with a significant difference between M and T

communities (Pseudo-F = 2.8242, unique permutations: 210, p(Monte Carlo) = 0.034). Moreover, PERMDISP test on condition showed no dispersion effect of sample (F= 0.79351, p(perm)= 0.3764).

Average similarity within condition, average dissimilarity among conditions (Table 4.9) and taxa major contribution to these (dis)similarities (Supplementary tables 5-6) were obtained through SIMPER analysis. The results indicated that there are more NIS in M than in T among the taxa most contributing to similarity (Supplementary table 5). In particular, the taxa contributing most (>10%) to the differentiation between communities of M and T panels were the bryozoan *Watersipora subtorquata* (d'Orbigny, 1852), the serpulid *Salmacina* sp. and the spirorbid *Janua* sp., which contributed with 31.13%, 22.28% and 12.04%, respectively (Supplementary table 6).

**Table 4.9 Average similarity within conditions and average dissimilarities between condition of experimental fouling communities in the Leghorn area.**

Average Similarity		Average Dissimilarity	
C	69.08	C & M	84.06
M	46.83	C & T	66.25
T	50.77	M & T	62.39

## 4.5 Discussion

In this study the capability of native communities to mitigate NIS colonization was assessed through two transplantation experiments in different study areas, namely the Gulf of La Spezia (2019) and the Leghorn coastal stretch (2020).

Donor sites resulted to be little or no colonized by NIS, an ideal condition for the transplantation experiments. Panels from these sites (C condition) showed generally lower values of all taxa cover in both study areas, probably due to factors characterizing the sites, including local currents, nutrients and plankton abundance, and propagule pressure, influencing the growth of fouling communities (Clark & Johnston 2009).

In marina sites, percent cover of taxonomic groups largely varied between the study areas, but both were dominated by Annelida and Bryozoa. A total of 11 NIS was found in La Spezia and 10 NIS in Leghorn, eight and seven of which belonging to one of the two dominant Phyla (Annelida or Bryozoa), respectively. In La Spezia area, *Branchiomma* sp., *H. elegans* and *S. plicata* resulted the NIS that colonized panels of all experimental conditions. Finally, in transplanted panels, a total of 7 NIS was found in La Spezia and 5 in Leghorn at the end of the deployment.

Considering the univariate analysis carried out with six different variables (Table 4.3 and 4.7), utilized to assess single aspects of biotic resistance, only two varied significantly between conditions (marina

vs. transplanted panels) in the two study areas. In the Gulf of La Spezia, “NIS/native species ratio” resulted different in the two conditions, highlighting a higher dominance of NIS in not-transplanted panels than in transplanted ones. On the other hand, no difference was highlighted between sites (i.e. marinas) and there was no interaction between factors (i.e. marina vs treatment), that means that the NIS/native species ratio could be considered site-independent (Quinn & Keough 2002). In Leghorn, the other study area, both “number of NIS” and “NIS/native species ratio” were significantly different between conditions, confirming that transplanted panels were less affected by NIS than not-transplanted panels.

In both areas, multivariate analyses showed that, after two months of immersion in a marina environment, fouling communities initially grown in distinct experimental conditions (marina vs. transplanted panels) resulted different from each other, and “NIS/native species ratio” (in both study areas) and “number of NIS” (in Leghorn study area) showed significative difference between conditions, indicating an effect due to transplantation and therefore suggesting biotic resistance. Moreover, NIS resulted among the taxa contributing most to panels grown and maintained in the marinas of both study areas, indicating a higher dominance of these species in non-transplanted panels. In multivariate analysis the highest dissimilarities among conditions were found between transplanted (low NIS contribution) and not-transplanted panels (high NIS contribution) in both study areas. Nevertheless, almost all NIS were recorded in the transplanted panels of both study areas, although with lower cover values than in non-transplanted panels.

Our experiment can be performed with the same experimental design in other study areas, in order to get more general information on the degree of biotic resistance by fouling communities grown in pristine environments. Furthermore, the same kind of the experiment could be repeated with an increased time period of panels deployment after the transplantation (three months or more). In this way, the further development of the communities in the different experimental condition could be assessed and NIS colonization success monitored in a longer time frame, considering that fouling communities show high seasonality (Lezzi et al. 2017).

The results showed in this study are similar to those from Gestoso et al. (2017). They found that fouling communities grown on panels in a MPA and then exposed to NIS propagule pressure in a touristic marina in Madeira showed lower NIS number and cover. In our experiments comparable results were shown in transplanted panels, and this can be considered another step in the studying of the role of communities rich in native species to contrast NIS invasions.

The results obtained in transplant experiments may indicate that biotic resistance by healthy and diverse communities living in MPAs should be able to minimize the presence of NIS in MPAs, but recent studies have also highlighted positive effects on NIS, due to the protection guaranteed by MPAs to every form of life. It seems indeed that MPAs can both protect against NIS and enhance invasion success, but this aspect is still poorly investigated and depends on the target species considered (Burfeind et al. 2013, Gallardo et al. 2017, Giakoumi & Pey 2017, Piazzini et al. 2021). The protection from NIS could occur due to richness and biomass growth of native species, that leave no niche available for other colonizers; while the facilitation could take place through other mechanisms, like the fishing restrictions inside the MPAs, which also include the unintended protection of NIS, and the higher diversity of predators and parasites of native species in MPAs (Burfeind et al. 2013).

In conclusion, these experiments provided additional information on the biotic resistance hypothesis, useful not only for a better understanding of the ecological processes, but also providing management indications for a better control strategy against NIS introduction in protected areas, as well as in ports. It is therefore advisable to continue the investigation and to establish a regular monitoring of fouling communities inhabiting MPAs or other sites of low impact level, evaluating the presence and abundance of NIS, as well as testing the biotic resistance of hard-bottom communities to NIS colonization. The outcomes of this study could be useful not only to better assess the role of native communities in the invasion process, but also to provide information on the need for the construction and implementation of nature-based or nature-mimicking coastal structures. Several of these “green” structures can be more suitable than standard artificial structures (e.g. concrete docks, tetrapods or plastic pontoons) for the recruitment of native species, with different success rate (Bulleri & Chapman 2010, Sella & Perkol-Finkel 2015, Morris et al. 2018, 2019, Airolidi et al. 2021), in order to partially compensate the environmental damage caused by port habitats (Firth et al. 2020).

## **5. Aim 3: Testing the predation effect on fouling communities**

### **5.1 Background**

In marine communities, the study of predation as an interspecific interaction has grown up since the pioneering experiments conducted by Robert T. Paine (Paine 1966, 1969, Lafferty & Suchanek 2016). More in detail, the first experimental approaches in the study of predation were applied on marine ecosystems and one of the most influential experiment was conducted by Paine (1966). His results suggested that local diversity is related to the number of predators in the food web and to their capability to prevent the monopolization of the limiting resources (i.e. the primary space in his experimental system) by single species (Paine 1966, Lafferty & Suchanek 2016).

Nowadays this interspecific interaction is known to have deep consequences on community structure and species diversity (Paine 1966, Freestone et al. 2011, Jurgens et al. 2017, Giachetti et al. 2019).

In the next decades, the study of predation (including grazing) blossomed and nowadays there are a lot of examples on the effect of the predation in marine ecosystems, including in coastal urbanized areas (e.g. Day & Osman 1981, Menge et al. 1986, Menge & Sutherland 1987, Brown & Swearingen 1998, Anderson & Connell 1999, Piazzini et al. 2000, Connell 2001a, Ferrario et al. 2016, Marić et al. 2016, Cheng et al. 2017, Jurgens et al. 2017, Hiebert et al. 2019). To give a few examples, Day & Osman (1981) discovered that in the offshore kelp forests across California the predation by a sea star species was able to speed up the successional process and to increase the bryozoan diversity, reducing the cover of a large dominant bryozoan species. Analysing the early colonization of seaweeds in New England, Lubchenco (1986) provided the first quantitative assessment of the role of both competition and predation. She found that the relative importance of both interactions depends on physical factors, relative sizes of predator and prey, and life history characteristics of the prey (Lubchenco 1986). As last example, an experiment about fish predation on sessile intertidal organisms (i.e., oysters) was conducted in an Australian oyster farm in 1999, and the results showed that fish altered the size distribution of oysters, although the authors suggested that predation may not have important long-term consequences on oyster natural populations (Anderson & Connell 1999).

More recently, the study of the predation has been integrated in the “biotic interactions hypothesis”, considered a potential driver of the “latitudinal diversity gradient” theory (Rodemann & Brandl 2017). The “latitudinal diversity gradient” is a well-established pattern in ecology, but the main driving mechanisms have been extensively debated, and the “biotic interactions hypothesis” is one of the prominent evolutionary theories that could explain this gradient (Rodemann & Brandl 2017). This “biotic interactions hypothesis” states that stronger interactions taking place closer to the equator



(including predation) caused increased rates of adaptation and speciation in the tropics, forming a “latitudinal diversity gradient” (Rodemann & Brandl 2017). Using a standardized methodology (the so-called “squidpops”) applied in several sites from Maine to Florida (USA), Rodemann & Brandl (2017) found a consistent latitudinal gradient in consumption pressure for a specific group of consumers (high predation at low latitudes and vice versa), therefore supporting the “biotic interactions hypothesis” as a potential driver of the “latitudinal diversity gradient” theory (Rodemann & Brandl 2017). As far as we know, in the Mediterranean Sea there are currently no broad scale studies with the aim to assess the consumption rates at different latitudes.

### **5.1.1 Predation on non-indigenous species**

Predation can have a role also in the interactions between NIS and native species. In general, NIS are exposed to strong environmental pressures when they arrive in a new region, and only those NIS able to prey on novel organisms, avoid novel predators, resist novel parasites and pathogens, and respond to new abiotic conditions can survive and establish self-sustained populations (Juetten et al. 2014, Papacostas & Freestone 2019, Pereira et al. 2019). On the other hand, the colonization by a new NIS can be facilitated by the absence in the new environment of both predators and parasites co-evolved with the NIS in the same original geographic range (Liu & Stiling 2006).

In recent years, several studies were carried out in order to assess the role of predation in NIS colonization success, although it is often difficult to elucidate the effective magnitude of this interaction. In a study regarding grazing, Cacabelos et al. (2010) focused on generalist herbivores, focusing on their feeding preferences between native seaweeds or a non-indigenous species in Northern Spain. These herbivores preferred grazing on native seaweeds than on the non-indigenous macroalga, leading to a putative facilitation for this NIS. Authors concluded that the spread and invasion of the NIS may be also facilitated by its intrinsic characteristics, like fast growth and high fecundity, and by local characteristics of the environment or of recipient assemblages (Cacabelos et al. 2010). Tomás et al. (2011) examined the role of *Sarpa salpa* (Linnaeus, 1758), a Mediterranean herbivorous fish, consuming three of the most invasive seaweeds of the Western Mediterranean, looking for any vertical (i.e., depth) and temporal grazing variation. Their results highlighted that *S. salpa* fed throughout the examined depth (5-35 m), concentrating in shallow waters and particularly grazing on a single NIS, *Caulerpa racemosa* (Forsskål) J. Agardh, 1873, providing a certain resistance to its invasion (Tomás et al. 2011).

As far as predation on non-indigenous invertebrates is concerned, the role of indigenous whelks was assessed in the regulation of the spread of a non-indigenous barnacle along the South African west coast (Robinson et al. 2015). The results indicated that the non-indigenous barnacle was mainly avoided by whelks. This phenomenon may be explained due to the shell of this NIS, thicker than the one of native barnacles. This means that predation-driven biotic resistance has not controlled the regional spread of this non-indigenous barnacle (Robinson et al. 2015). Another case study investigated the predation by native species on a non-indigenous crab, *Percnon gibbesi* (H. Milne Edwards, 1853), inside and outside a Marine Protected Area (MPA) in Italy (Noè et al. 2018). The authors observed a higher predation rate on crabs in protected than unprotected sites, suggesting that the diversity and abundance of native predators mitigate the success of *P. gibbesi*. Moreover, the authors provided experimental evidence that the restoration of predator assemblages provide 'biotic resistance' to MPAs against *P. gibbesi* invasion (Noè et al. 2018).

Finally, predation on non-indigenous fishes by native ones was reported. Giakoumi et al. (2019) assessed the predation rate on native and non-indigenous fish species in Mediterranean protected and unprotected areas. They investigated if high-level predatory fish populations within Mediterranean MPAs can exert top-down control on non-indigenous fishes, tethering and exposing inside and outside MPAs dead specimens of both native, *Sardina pilchardus* (Walbaum, 1792) and *Boops boops* (Linnaeus, 1758), and NIS - *Siganus rivulatus* Forsskål & Niebuhr, 1775. Although no significant differences were observed in the consumption of non-indigenous or native fishes between areas, more high-level predators interacted with the tethered fish inside the MPAs than in unprotected areas. Their results suggest a potential biocontrol of non-indigenous fishes by high-level predators (Giakoumi et al. 2019).

Unfortunately, few studies on more complex food webs were conducted. An example was provided by Marić et al. (2016). Using stable isotope analysis, they investigated trophic interactions between indigenous benthic taxa and NIS on Lampedusa Island (Italy). They assessed trophic positions of species involved and isotopic niches of consumers, quantifying the food source contribution to diets of indigenous and non-indigenous herbivores. No niche overlap between NIS and native macroinvertebrate and fish was highlighted, and they conclude that, as an additional food source, the non-indigenous alga *Caulerpa cylindracea* Sonder, 1845 is increasing the diversity of available prey and might facilitate the expansion of other herbivorous NIS (Marić et al. 2016).

Furthermore, food webs in lower latitudes can have strong consumer pressure and top-down control due to their trophic complexity, thus they could be more robust to alterations by NIS than higher latitude food webs, that generally show less complexity (Papacostas & Freestone 2019). More in

general, increased predation in tropics could explain the lower success of NIS colonization at low latitudes (Freestone et al. 2011, 2013, 2021), although further experiments are required to confirm these and other hypotheses on the role of biological interactions in NIS success (Wells & Bieler 2020).

### **5.1.2 Influence of predation on fouling communities**

In coastal urbanized habitats, where artificial structures flourish, fouling communities can dominate the ecological landscape (Albano 2019). In these novel ecosystems the ecological interactions like predation have been poorly studied too in the Mediterranean Sea (e.g. Ferrario et al. 2016), but several local-scale studies on the role of predation in other marine regions, including the effects on NIS, have been carried out since the mid 90's (see also Osman & Whitlatch 1995, Brown & Swearingen 1998, Nydam & Stachowicz 2007, Epelbaum et al. 2009, Gestoso et al. 2018, Hiebert et al. 2019, Giachetti et al. 2020, Dias et al. 2020).

The first issue to be assessed is if, in the urbanized systems, consumers assemblages can be comparable to those from low-impacted and pristine ecosystems. Some studies on the richness and abundance of ichthyofauna in urbanized and not-urbanized ecosystems showed different scenarios. In the Western Mediterranean Sea, it seems that fish assemblages associated with exposed and sheltered sides of breakwaters significantly diverged from those associated with adjacent sandy habitats, but not from those associated with adjacent rocky shores (Guidetti 2004, Clynick 2006). Moreover, a study in the South-West Atlantic showed that urbanized habitats cause microspatial modifications to fish assemblages, creating conditions different from those in "natural" habitats (Pastro et al. 2017). Broader-scale research conducted in a latitudinal gradient in the Western Atlantic (about 9°- 44° N) on cryptic benthic fishes found that the biodiversity patterns from dock pilings followed a spatial gradient and, above all, fish assemblages from dock pilings were less diverse and had lower densities than nearby reef habitats (Brandl et al. 2017). Lastly, along Eastern US coast it was discovered that daily rate of consumption by fish assemblages were consistently higher in not-urbanized areas than in urbanized ones (Rodemann & Brandl 2017).

Specifically considering the role of predation in shaping fouling communities, in the last decades few experiments were conducted with settlement panels and cages for predator exclusion. Nowadays predation is considered a strong driver of the development of communities, but the importance given to predation could also be influenced by under-reporting of studies that fail to support its role, due to a scientific culture of rejecting "negative" results in marine ecology (Connell 2001a).

In the following section, several examples of studies on predation on fouling communities around the globe are showed, underlining both major and minor effects of predation in determining the composition of these assemblages.

In New England (USA), the predation on ascidians by two small gastropods species was investigated, finding that they affected recruitment throughout the entire reproductive season (Osman & Whitlatch 1995). In the Gulf of Mexico, Brown & Swearingen (1998) found that predators, like crabs or gastropods, seem to regulate barnacle abundance. On the contrary, in Sidney Harbour, the results revealed no differences among fouling assemblages on caged and uncaged plates, highlighting that predation may be intense but without an ecological role in shaping the community structure (Connell 2001a). In an experiment carried out in Connecticut (USA) it has been observed an effect of predation on recruitment and young communities composition, although predators had little or no effect on the adults (Osman & Whitlatch 2004). The effect of two molluscan predators (i.e., a chiton and a limpet) on the recruitment of sessile invertebrates was examined in an experiment held along Californian coasts, highlighting a considerable impact of these predators in determining the community composition (Nydham & Stachowicz 2007). In southeastern Brazil, Vieira et al. (2012) found that the localized extinction of ascidians in predation treatments was compensated by an increase in the diversity of bryozoans and barnacles. Moreover, predation during the early stages of community development had only short-term effects on taxa richness and composition (Vieira et al. 2012). In an Australian experiment, the overall structure of the communities was found divergent due to predation, with bryozoans and hydroids occupying more space on uncaged panels, and the predation effect was consistent through time; this study demonstrated that strong predation effects on a particular taxon was able to alter the overall community composition (Lavender et al. 2014). Rico et al. (2016) carried out a study in southern Argentina coast and found no differences in species richness between caged and uncaged panels, but higher diversity of communities grown on uncaged panels. Moreover, uncaged panels were dominated by algae, indirectly promoted by large predators (Rico et al. 2016). An interesting research conducted in south Brazil focused on the role of predation on colonial and solitary ascidians (Hiebert et al. 2019). The authors find that predation influence more growth and survivorship of colonial than solitary ascidians and their hypothesis is that colonial ascidians are nevertheless advantaged, because they can regrow when partially consumed, adjusting in shape and space to grow into crevices (Hiebert et al. 2019). One of the most comprehensive studies of fouling communities and their predators across latitudes was conducted by Freestone et al. (2021), with the aim to assess if the ecological interactions strengthen toward lower latitudes. They confirmed that predation was stronger, reduced prey biomass and altered prey composition at low latitudes, and no effects were reported at high latitudes (Freestone et al. 2021).

Finally, another interesting aspect to consider is the role of predation on NIS, an important component of fouling communities in ports, and if it can act as “biocontrol” to mitigate the establishment and spreading of NIS. Several studies around the world investigate the effect of predation in regulating NIS spreading, with contrasting observations on the effect of native predators on NIS (a useful summary can be found in Giachetti et al. 2020 , Table 1). In particular, several studies were conducted in Western Pacific Ocean (Forrest et al. 2013, Astudillo et al. 2016, Yorisue et al. 2019), Eastern Pacific (Epelbaum et al. 2009, Dumont et al. 2011b a, Simkanin et al. 2013, Needles et al. 2015, Rogers et al. 2016, Leclerc 2020) Western Atlantic Ocean (Kremer & da Rocha 2016, Giachetti et al. 2019, 2020, Dias et al. 2020), and Eastern Atlantic (Gestoso et al. 2018, Dias et al. 2020, Gauff et al. 2022). Among these studies, Kremer & da Rocha (2016) found an effect only on some taxonomic groups; Astudillo et al. (2016) found a seasonality effect that obscured potential predation effect; Needles et al. (2015), Gauff et al. (2022), and Gestoso et al. (2018) found an increase of NIS colonization due to native predation in certain circumstances. On the other hand, NIS colonization resulted reduced in the other experiments (namely, Epelbaum et al. 2009, Dumont et al. 2011b a, Simkanin et al. 2013, Forrest et al. 2013, Rogers et al. 2016, Yorisue et al. 2019, Giachetti et al. 2019, 2020, Leclerc 2020), including Dias et al. (2020) that found a strong effect only in tropical regions.

At the moment, only three experiments were conducted in European Seas, all in the Atlantic Ocean (Leclerc & Viard 2018, Dias et al. 2020, Gauff et al. 2022), but no comparable experiments were carried out in the Mediterranean Sea.

## **5.2 Aim of the study**

Inspired by the works briefly mentioned in the previous section, in this study was performed for the first time in the Mediterranean Sea —namely in the Gulf of La Spezia (Ligurian Sea, Italy)— a manipulative experiment using PVC panels and plastic cages to assess the role of macro predators in the development of fouling communities in a marina. A difference in the composition of fouling communities from caged and uncaged panels is expected, as reported in literature. More in detail, a dominance of calcareous species is expected in uncaged panels. We would also assess if NIS established in one Italian site can be affected by predation or not.

## **5.3 Materials and methods**

This experiment was conducted in Santa Teresa Bay (ST: 44.081544°N – 9.881881°E; Figure 5.1) from August 2020 to October 2020. Santa Teresa Bay is a little embayment in the Gulf of La Spezia

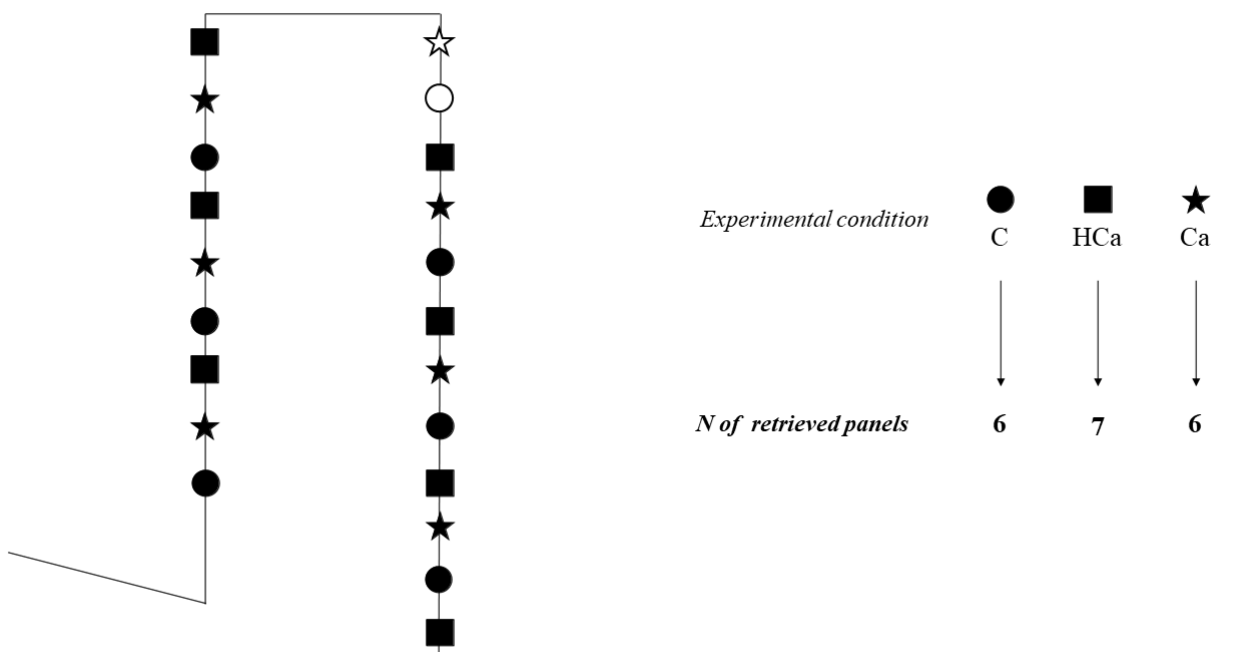
designed to host few recreational boats on a single floating pontoon, and known to present a high incidence of NIS in fouling communities (Aim 1, see section 3 and Tamburini et al. 2021), probably due to the presence of a close aquaculture facility (Rius 2011). The role of predation in the development of fouling communities, and in particular on NIS settlement, was investigated through a manipulative experiment, using PVC panels (following the SERC protocol, see Paragraph 2.3) and plastic cages to not allow the access to panels by macro predators.



**Figure 5.1** Satellite image of the selected site (red arrow) in Santa Teresa Bay (ST). Graphical elaboration from Google Earth Pro.

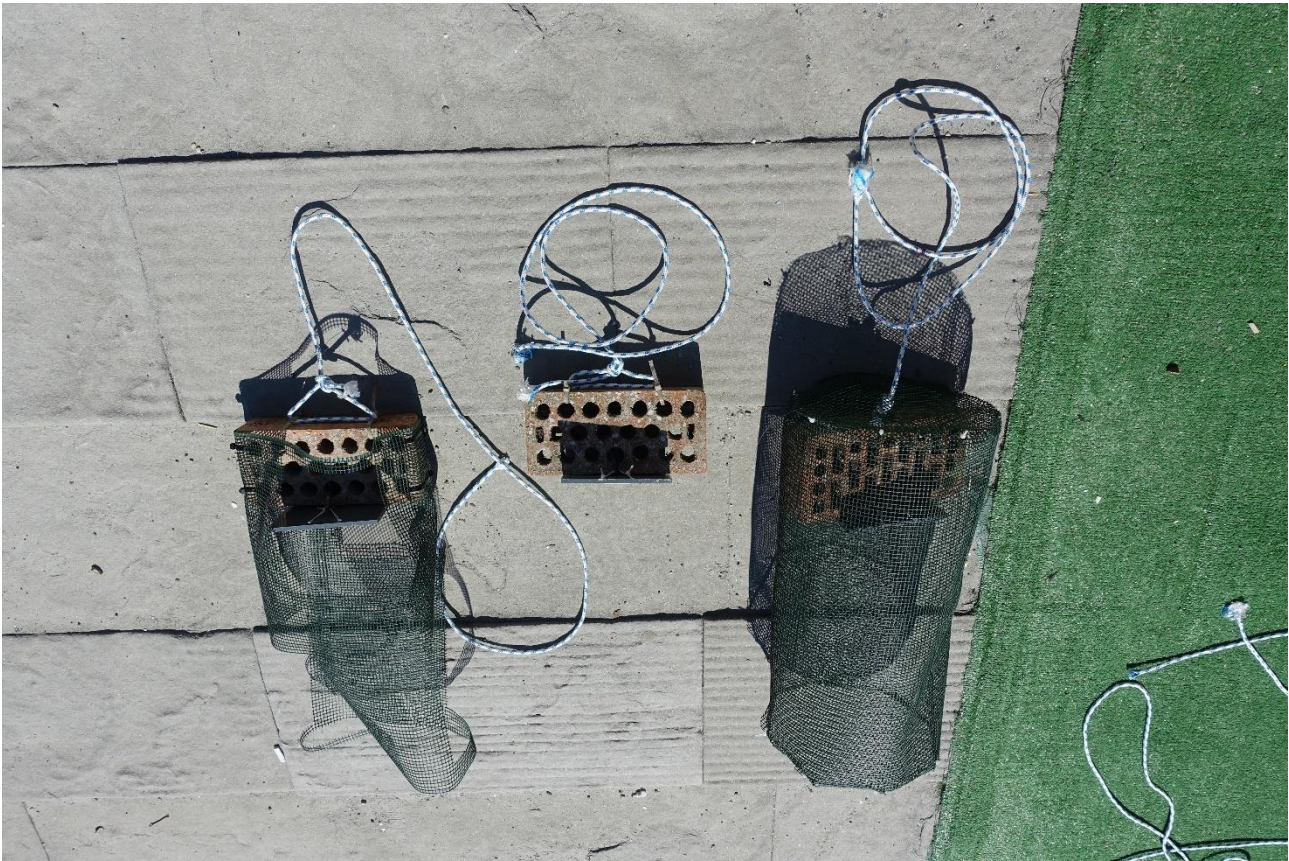
For this study, experimental units composed by panels and bricks and associated plastic cages were hang on the only floating pontoon of the bay (Figure 5.1, 5.2), in order to provide similar environmental conditions to all the panels. Twenty-one panels were divided in three experimental conditions (seven per condition, but two panels were lost due to external causes; see Figure 5.2): “control panels” (C), “half-caged panels” (HCa) and “caged panels” (Ca), in order to assess the predation effect on the fouling assemblages growing onto the panels (Figure 5.2, 5.3). The condition HCa was included as control of the artifacts - i.e. cages - (Steele 1996). Cages were green plastic cylinders, about 25 cm in diameter and 50 cm in height, with a mesh size of about 0.5 cm, fixed to bricks through plastic cable ties.

A GoPro® camera was deployed in the water during the cage cleaning days and during the retrieval of panels, for about four hours in the late morning, in order to qualitatively evaluate macropredator presence and activity through time-lapse photography (one photo each 30 seconds).



**Figure 5.2** Design of the cage experiment deployed. On the left side of the picture, panels disposal along the floating pontoon is shown. This pattern of panels disposal was applied to ensure similar environmental condition to all experimental treatments. Empty symbols represent panels lost during exposure. The realized experimental design is shown on the right side of the picture. C: control panels (i.e. uncaged); HCa: half-caged panels; Ca: caged panels.

All panels were immersed for a total of 70 days, from August to October 2020. All the cages were manually cleaned with brushes after 28 and 55 days from deployment, in order to allow water circulation inside cages. At the end of the experiment, all panels were retrieved, weighted, photographed and preserved in plastic bags with ethanol 70%. In the laboratory, all panels were observed under dissecting microscopes for taxonomic identification of sessile invertebrates. The percent cover of the whole fouling assemblage was assessed by point count method (n=50 points), that allows the multiple count of different taxa on the same point (i.e. the total cover can exceed 100%), following the SERC protocol; for details see section 2.3) (Chang et al. 2018).



**Figure 5.3** The three experimental condition of the experiment. From the left: half-caged panel (HCa), control panel (C) and caged panel (Ca). Panels were deployed as seen in figure 2.3, with the experimental surface facing downward. Picture by the author.

For each panel, net wet weight, species richness (S), NIS/native species ratio (Olenin et al. 2010) and percent cover of each taxon were calculated, considering only the sessile macrozoobenthos. The net weight was measured to assess if predation could have influence on the total biomass of assemblages. To each taxon a biogeographic status (sensu Chapman & Carlton 1991) was assigned, namely NIS, native species (including cryptogenic species - i.e. species that cannot be considered either native or introduced due to lack of information) or unresolved taxa (due to insufficient taxonomic resolution e.g. damaged specimens or juveniles). Cryptogenic species were included in the group of native species following a conservative approach. Differences in mean values of weight, S, NIS/native species ratio, Shannon Index ( $H'$  based on  $\log e$ ) and percent cover of native species, NIS and the whole fouling assemblage were assessed by one-way ANOVA tests (fixed factor: “condition” with three levels: C, HCa, Ca; unequal sample size; Figure 5.2). In all the cases of non-homogeneity of the variances, data were properly transformed, and a more conservative critical value ( $\alpha < 0.01$ ) was chosen if the data transformation was not successful.

Non-metric multi-dimensional scaling (nmMDS) among samples (square-root transformation; resemblance matrix obtained using Bray-Curtis similarity) was used to visualize the pattern of fouling

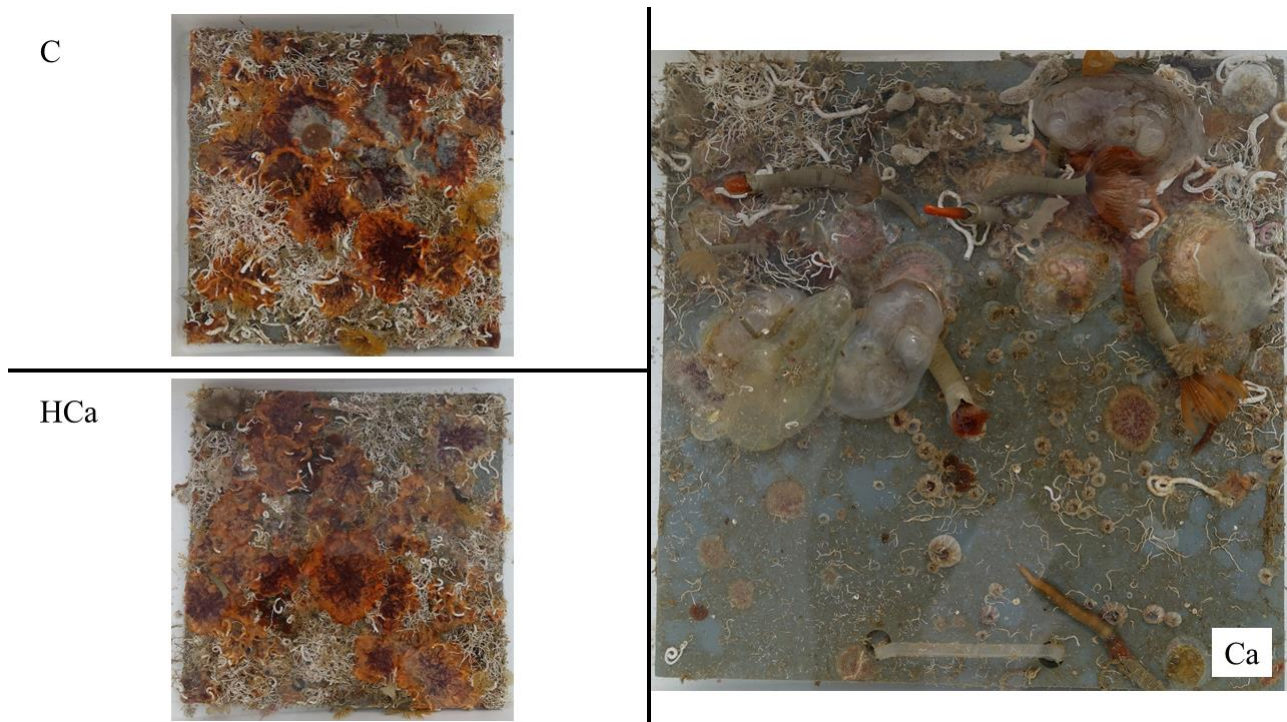


communities of experimental panels at the end of the experiment. Cluster analysis (cluster mode: group average) was used to assess similarity among samples. PERMDISP test and one-way PERMANOVA (Anderson et al. 2008) were carried out to test differences in community composition within and between factor “condition” (fixed, three levels: C, HCa, Ca). Moreover, SIMPER analyses among experimental conditions was performed. All the analyses were carried out with the software Microsoft Excel, R (R Core Team 2017) and PRIMER 6 with PERMANOVA+ add-on package (Clarke & Gorley 2006, Anderson et al. 2008).

## 5.4 Results

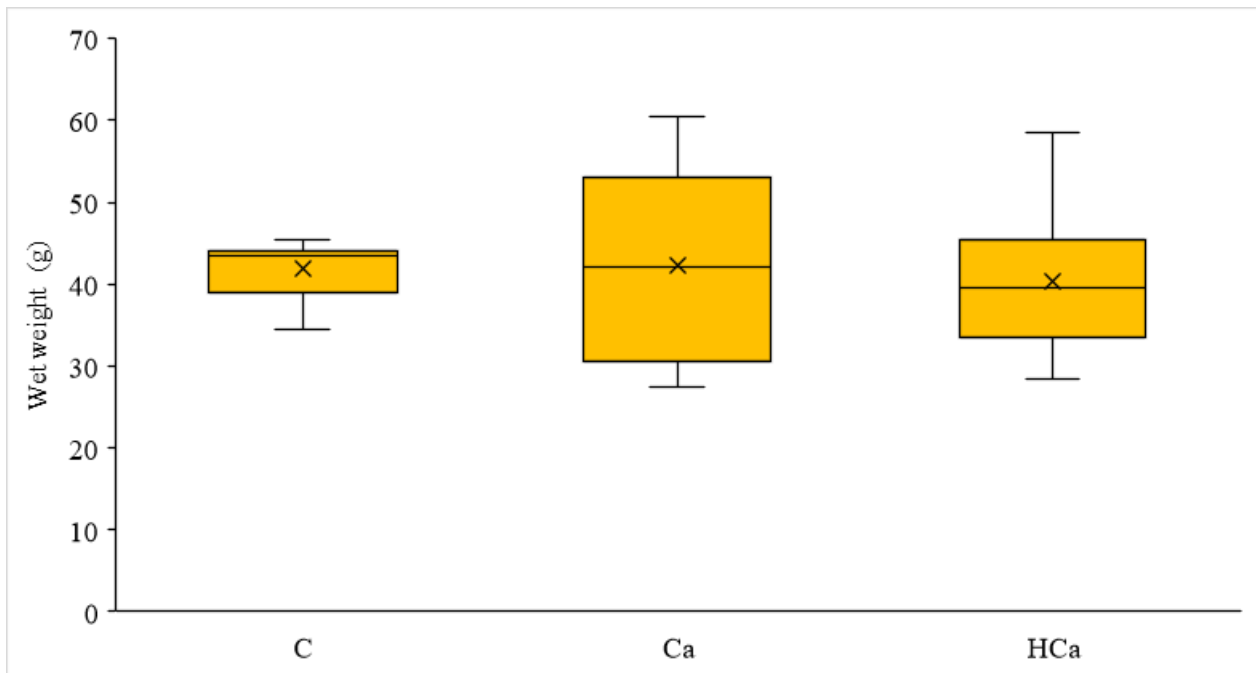
A total of 395 GoPro® photos taken automatically under the floating pontoon was obtained during the experiment, and two (native) fish species of potential macro predators were identified: the Osteichthyes *Oblada melanura* (Linnaeus, 1758) and *Sarpa salpa* (Linnaeus, 1758). Moreover, during the retrieval of panels, a sea urchin, probably *Arbacia lixula* (Linnaeus, 1758), was found grazing on one panel.

Panels from C and HCa showed a dominance of calcareous species (especially bryozoans), while Ca panels were more dominated by “soft” species (Figure 5.4).



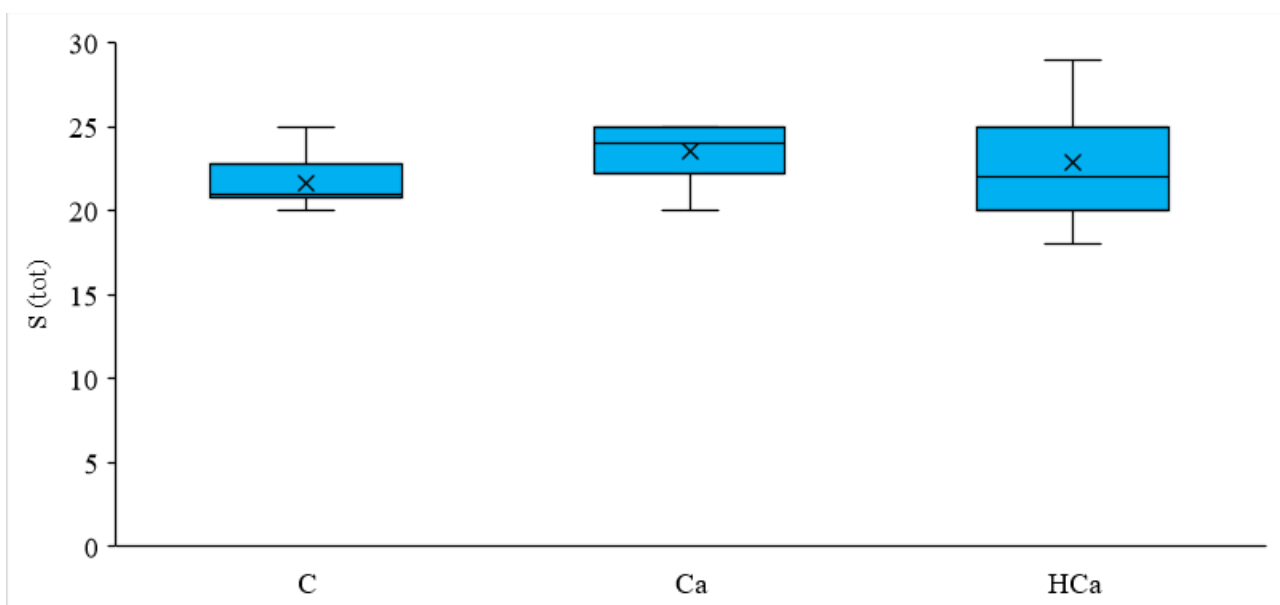
**Figure 5.4** Example of panels from the three conditions. C: control panels (i.e. uncaged); HCa: half-caged panels (i.e. artifact control); Ca: caged panels.

The mean panel wet weight was  $42 \pm 4$  g in C,  $42 \pm 12$  g in Ca and  $40 \pm 10$  g in HCa condition, and no significant difference was highlighted (Figure 5.5; Table 5.1).



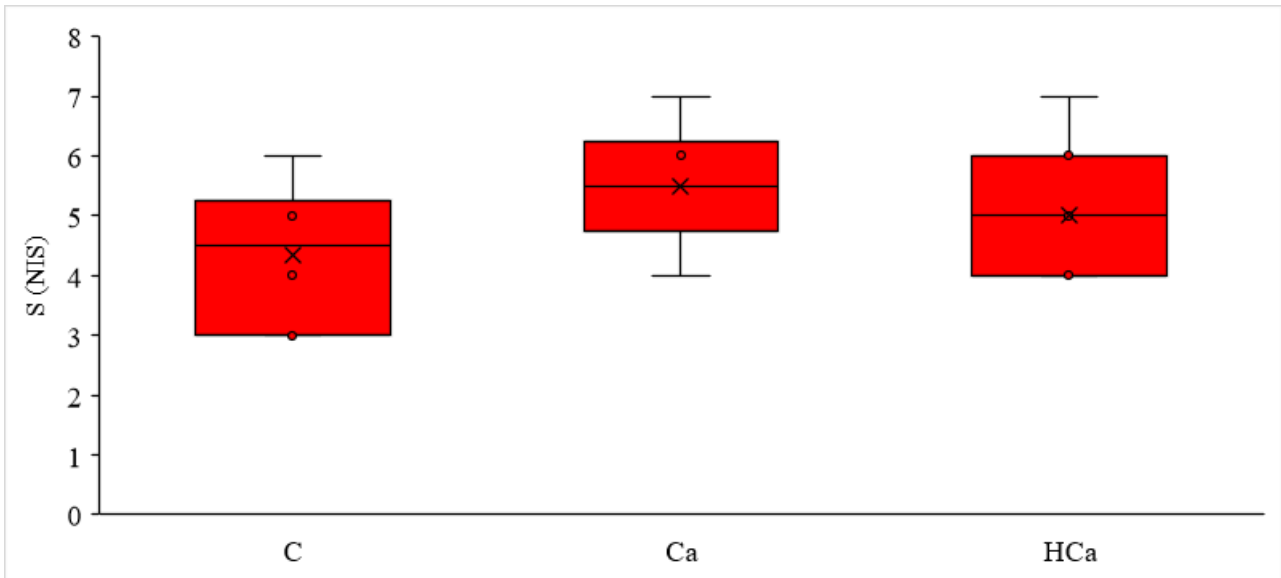
**Figure 5.5** Boxplots of panel wet weights (g) in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

A total of 46 taxa belonging to seven taxonomic macrogroups (including nine NIS) was found on all panels, independently from the experimental condition (Supplementary table 7). Mean total species richness resulted similar among conditions, with  $21.7 \pm 1.8$  in C,  $23.5 \pm 1.9$  in Ca and  $22.9 \pm 3.7$  in HCa, and no significant difference were observed (Figure 5.6; Table 5.1)



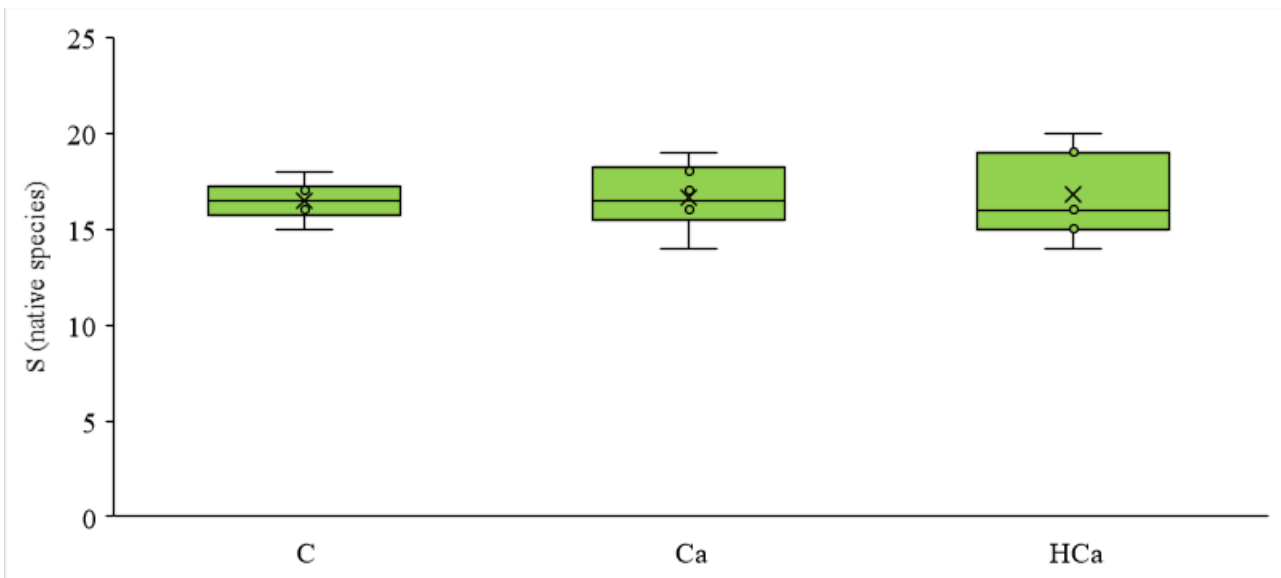
**Figure 5.6** Boxplots of total species richness (S) in the three experimental conditions. “x” in the plots indicate mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

NIS mean richness was not statistically different among conditions, although it was  $4.3 \pm 1.2$  in C,  $5.5 \pm 1.0$  in Ca and  $5.0 \pm 1.2$  in HCa (Figure 5.7, Table 5.1).



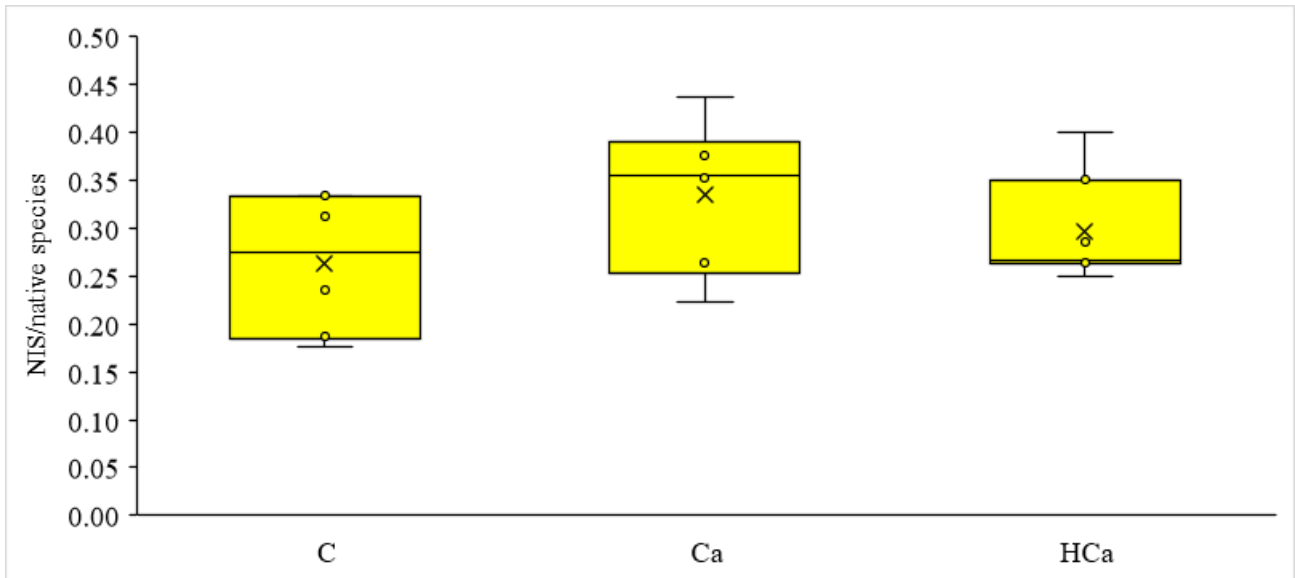
**Figure 5.7** Boxplots of NIS richness in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

Mean richness of native species was not significant different too (Table 5.1). Mean values were  $16.5 \pm 1.0$  (C),  $16.7 \pm 1.8$  (Ca), and  $16.9 \pm 2.4$  (HCa) (Figure 5.8).



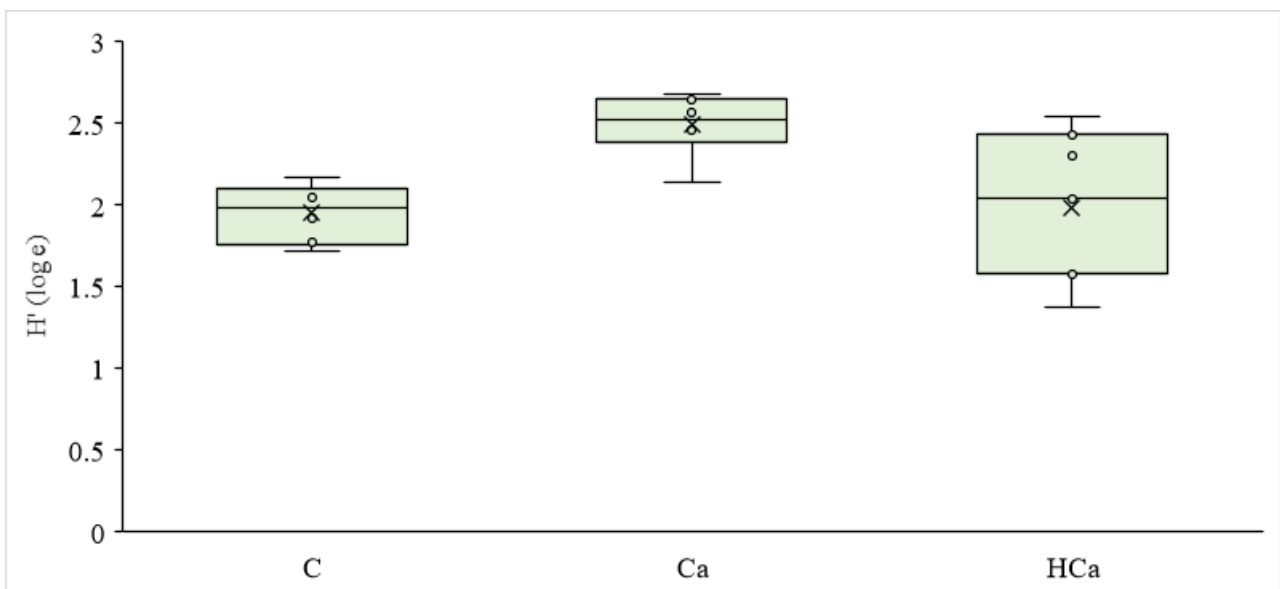
**Figure 5.8** Boxplots of native species richness in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

The mean ratio between NIS and native species was the highest in Ca panels ( $0.33 \pm 0.08$ ), followed by HCa ( $0.30 \pm 0.06$ ) and C ( $0.26 \pm 0.07$ ), and no difference was highlighted by ANOVA test also for this variable (Figure 5.9; Table 5.1)



**Figure 5.9** Boxplots of NIS/native species ratio in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

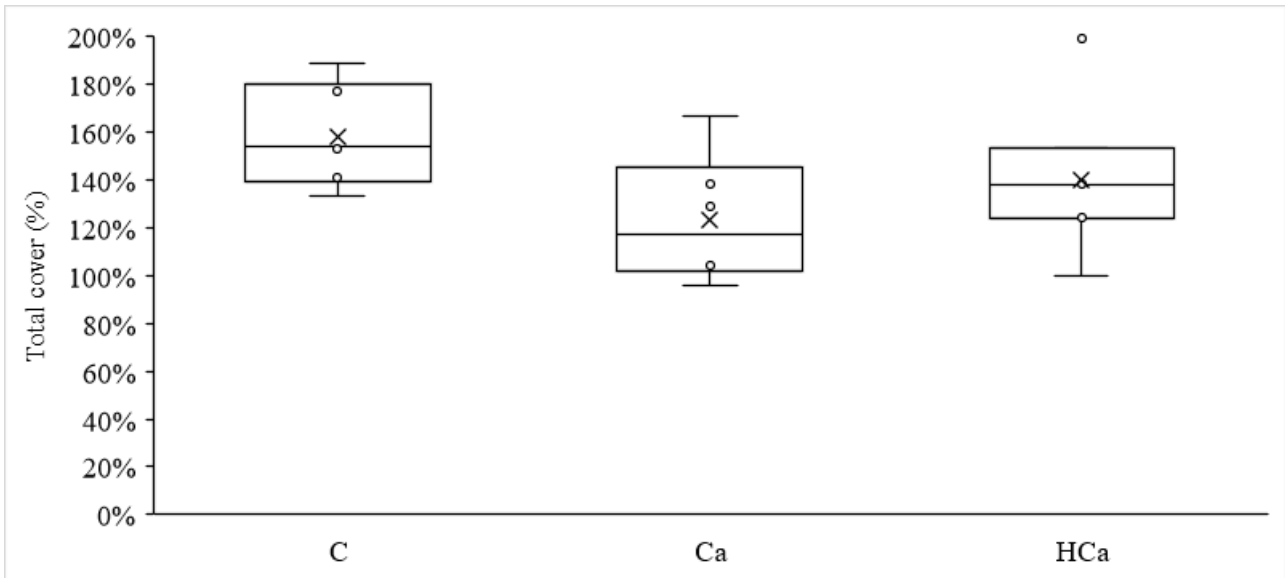
The Shannon Index ( $H'$ ) was significantly different among conditions, namely  $1.95 \pm 0.18$  in C,  $2.49 \pm 0.20$  in Ca and  $1.98 \pm 0.46$  in HCa (Figure 5.10; Table 5.1), in particular it was higher in Ca than in C ( $p\text{-adj} = 0.025$ ) and in HCa ( $p\text{-adj} = 0.029$ ).



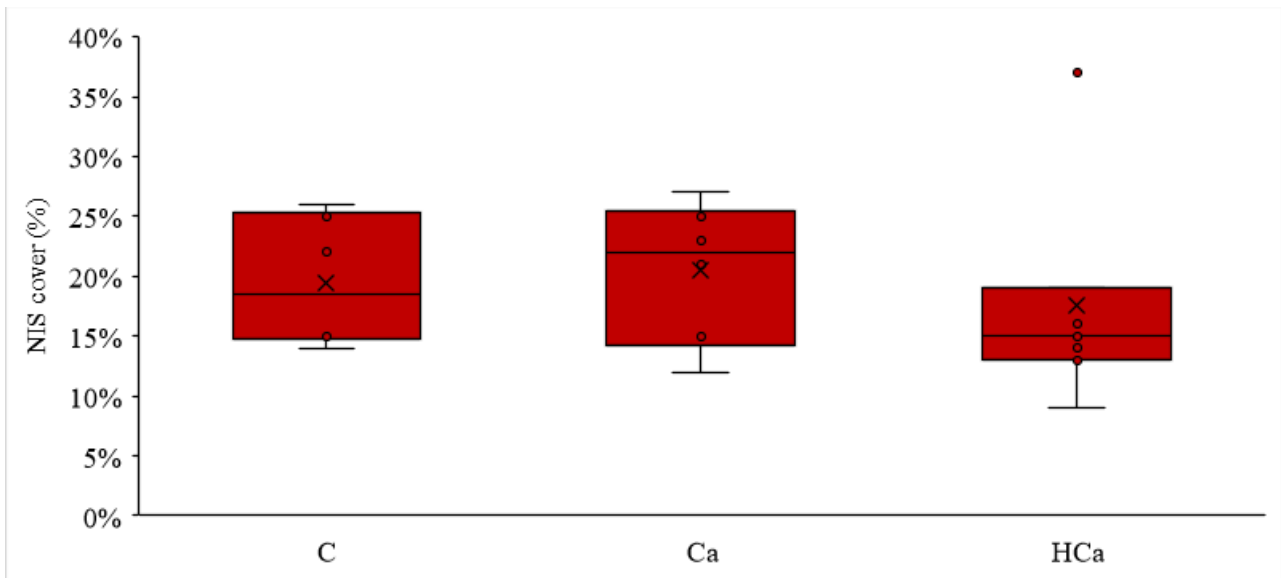
**Figure 5.10** Boxplots of the Shannon Index ( $H'$ ) in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

Mean total cover of fouling assemblages was similar in the three conditions,  $158 \pm 21\%$  in C,  $123 \pm 27\%$  in Ca and  $140 \pm 31\%$  in HCa, respectively (Fig 5.11; Table 5.1); as well as NIS cover was similar among conditions, namely  $20 \pm 5\%$  in C,  $21 \pm 6\%$  in Ca and  $18 \pm 9\%$  in HCa (Fig 5.12; Table 5.1). On the other hand, mean cover of native species was significantly lower in Ca ( $103 \pm 22\%$ ) than in C

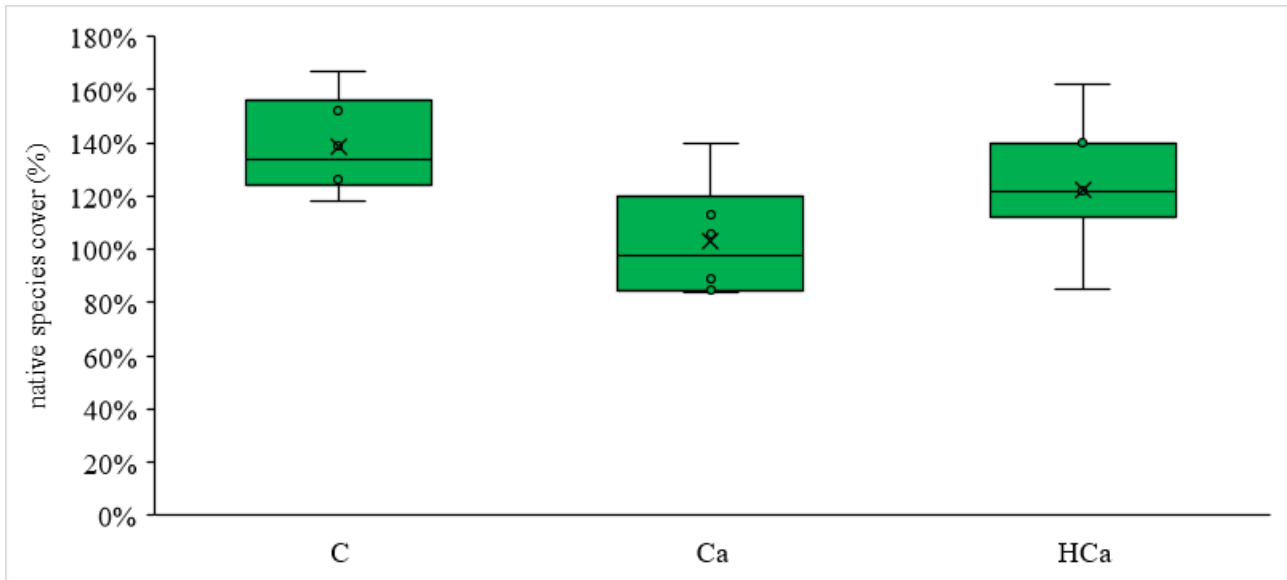
( $139 \pm 18\%$ ;  $p\text{-adj} = 0.03$ ), but not significantly lower than in HCa ( $123 \pm 24\%$ ;  $p\text{-adj} = 0.26$ ) (Figure 5.13; Table 5.1).



**Figure 5.11** Boxplots of the total panel cover in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.



**Figure 5.12** Boxplots of NIS cover in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.



**Figure 5.13** Boxplots of native species cover in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

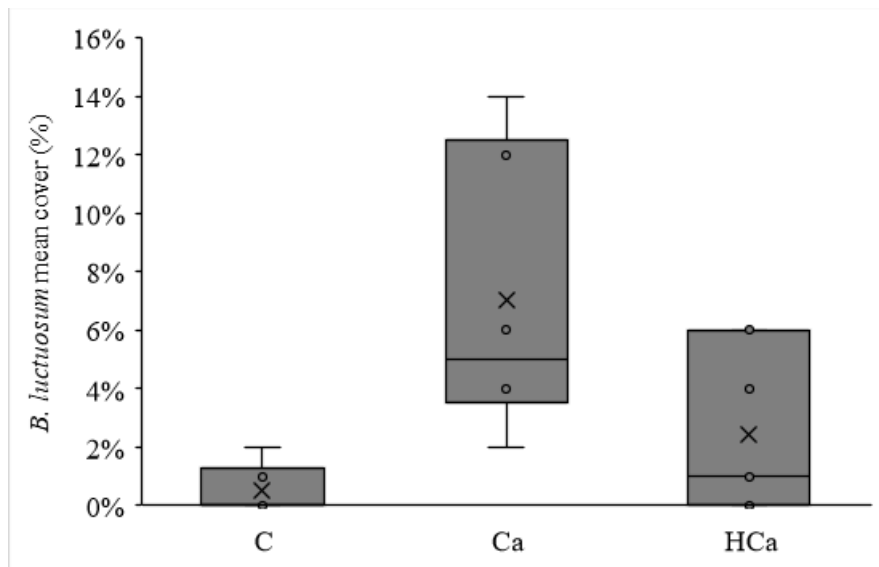
**Table 5.1** Summary of the ANOVA tests on the selected variables. Significant *p* values are in bold.

<b>Variable</b>	<b>F value</b>	<b><i>p</i></b>
Wet weight (g)	0.0792	0.9242
S (tot)	0.7202	0.5018
S (NIS)	1.5798	0.2365
S (native species)	0.0596	0.9424
NIS/native species	1.6188	0.229
H' (log e)	5.5412	<b>0.01484</b>
Total cover (%)	2.4976	0.1138
NIS cover (%)	0.2848	0.7559
native species cover (%)	4.1098	<b>0.03628</b>

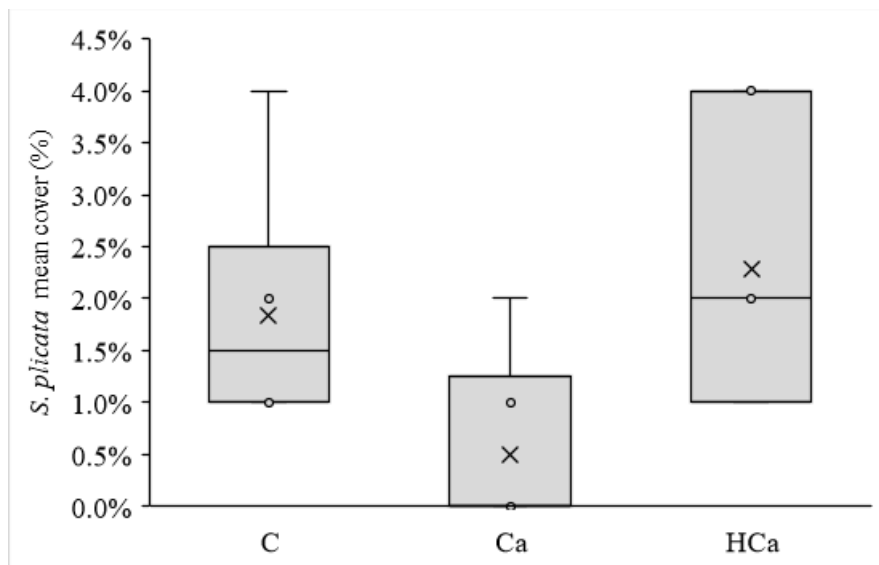
Regarding single NIS, differences of mean cover among conditions were tested only for species with a total mean above 1%: for this reason, ANOVA tests (Table 5.2) were conducted only on *Branchiomma* sp., *Branchiomma luctuosum* (Grube, 1870), *Hydroides elegans* (Haswell, 1883) and *Styela plicata* (Lesueur, 1823). *B. luctuosum* cover (Figure 5.13) was significantly different among conditions (Table 5.2), in particular it was higher in Ca than in C ( $p$ -adj= 0.004) and in HCa ( $p$ -adj= 0.04). Also *S. plicata* cover (Figure 5.14) was different among conditions (Table 5.2), more in detail it was statistically lower in Ca than in HCa ( $p$ -adj= 0.03), but no significant difference was highlighted between C and Ca panels ( $p$ -adj= 0.13).

**Table 5.2 Summary of the ANOVA tests on NIS with total mean cover above 1%. Mean cover is among all plates, regardless experimental condition.**

NIS (mean cover > 1%)	mean cover	F value	p
<i>Branchiomma</i> sp. (Tamburini et al. 2021)	2.8%	0.4316	0.6568 ns
<i>Branchiomma luctuosum</i> (Grube, 1870)	3.2%	7.7513	0.0044 **
<i>Hydroides elegans</i> (Haswell, 1883)	9.7%	2.1297	0.1513 ns
<i>Styela plicata</i> (Lesueur, 1823)	1.5%	4.4003	0.0300 *



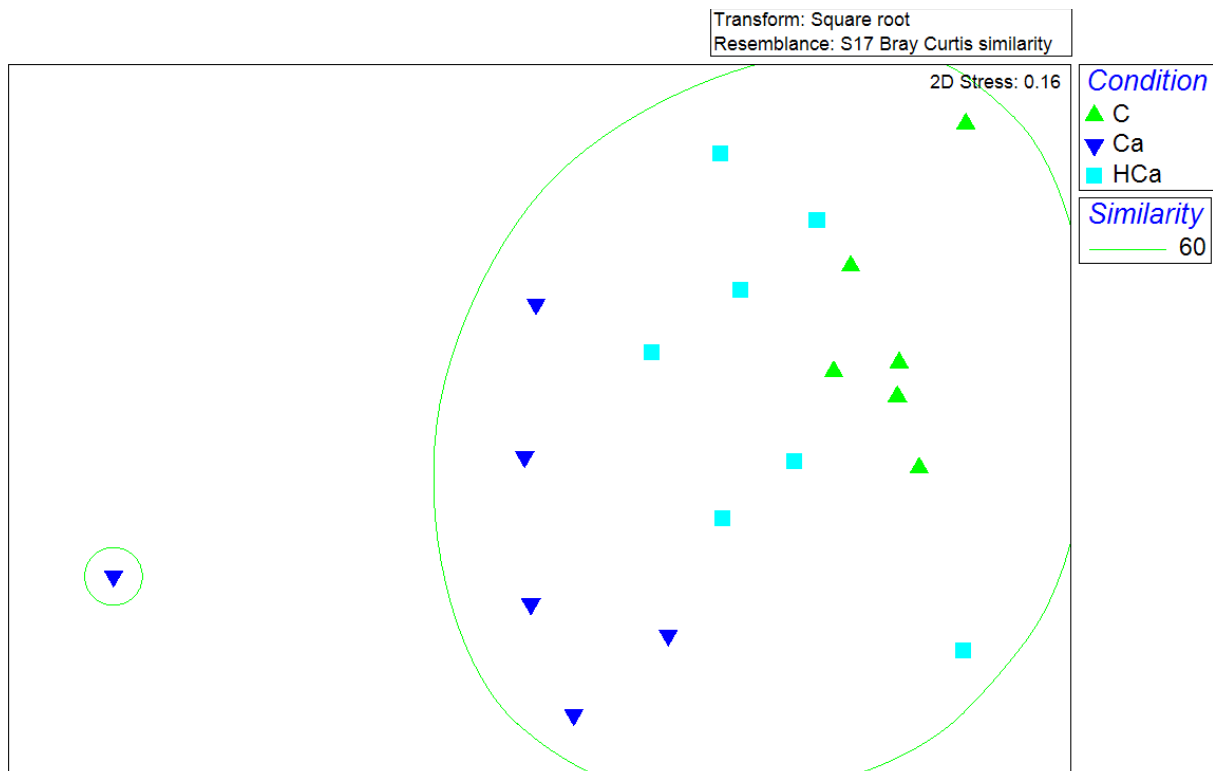
**Figure 5.14** Boxplots of *B. luctuosum* cover in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.



**Figure 5.15** Boxplots of *S. plicata* cover in the three experimental conditions. “x” in the plots indicates mean values. Abbreviations of experimental conditions are indicated in Figure 5.4.

Regarding multivariate analyses, nmMDS showed similarity in fouling assemblages of the same condition and a clear separation among assemblages from different condition (Figure 5.15). As

expected, C and HCa assemblages resulted more similar to each other than C and Ca assemblages (Figure 5.15). The overlapped cluster analysis indicated at least 60% similarity among all samples, except for one sample of Ca condition (Figure 5.15).



**Figure 5.16 nmMDS on Bray Curtis similarity (square root transformed data) among panels of C, Ca and HCa conditions. Green circles indicate the boundary of 60% similarity, provided by Cluster Analysis. Abbreviations of experimental conditions are indicated in Figure 5.4.**

PERMDISP test showed homogeneity of multivariate dispersion ( $F= 2.2$ ,  $p$  (perm)= 0.2286), while PERMANOVA main test showed a significant difference among assemblages from different conditions (Pseudo- $F= 2.7007$ ;  $P$ (perm)= 0.0004; Unique perm= 9918). In particular, post-hoc pairwise tests showed a difference in the fouling assemblages of C and Ca ( $t= 2.175$ ;  $P$ (MC)= 0.0022), and of HCa and Ca ( $t= 1.5962$ ;  $P$ (MC)= 0.0317), but no difference was highlighted between C and HCa assemblages, as expected ( $t= 0.90251$ ;  $P$ (MC)= 0.5512).

Lastly, SIMPER analysis revealed the contribution of the single taxon to the similarity within conditions and to the dissimilarity among them (Supplementary table 8-9). More in detail, C assemblages showed the highest similarity (average similarity: 71.57), followed by HCa (average similarity: 66.75) and Ca (average similarity: 63.08). NIS most contributing to this similarity were *Hydroides elegans* (Haswell, 1883) in C (contribution: 9.96%) and in HCa (contribution: 7.68%), while it was *B. luctuosum* in Ca (contribution: 6.78%) (Supplementary table 8). Speaking on native taxa contribution, *Schizoporella errata* (Waters, 1878) was the most contributing species in C, Ca



and HCa, with a contribution of 24.36%, 11.87%, and 19.83%, respectively. *S. errata* contribution was followed by *Salmacina* sp. contribution in C (13.32%) and HCa (10.07%), and by *Anomia ephippium* Linnaeus, 1758 contribution in Ca (9.40%) (Supplementary table 8). Regarding dissimilarity among conditions, the highest average dissimilarity was between C and Ca (42.28), followed by Ca-HCa (38.93) and C-HCa (30.79), as expected. NIS most contributing to dissimilarity were *B. luctuosum* for C-Ca (contribution: 5.61%) and for Ca-HCa (contribution: 4.79%), and *H. elegans* for C-HCa (contribution: 4.58%) (Supplementary table 9). Moreover, native taxa most contributing to dissimilarity was *S. errata* for C-Ca dissimilarity (contribution: 9.7%) and for Ca-HCa (12.13%), and *Salmacina* sp. for C-HCa (8.43%) (Supplementary table 9).

## 5.5 Discussion

In this caging experiment the influence of predation on fouling assemblages was investigated, also focusing on its effect on NIS. Our results constituted a further contribute on the role of predation as a driver of the development of fouling communities, focusing on early stages.

First of all, two fish species were identified – *O. melanura* and *S. salpa* - through image analysis. *O. melanura* is a very common species in the Mediterranean Sea and can be considered an opportunistic predator (Pallaoro & Jardas 2003, Fernandez-Jover & Sanchez-Jerez 2015). Its diet depends both on season and on specimen size, and can include crustaceans, copepods and several benthonic and pelagic species. For this reason, it can be considered a potential predator of the experimental fouling assemblages. On the other hand, *S. salpa* is generally considered an herbivorous fish (Havelange et al. 1997), but juveniles seem to eat also crustaceans, including copepods (Dobroslavic et al. 2012). Its role in the predation of fouling communities is therefore dubious. It was not possible to record any other predator interacting with the experimental systems, including crabs or sea urchin (Nydam & Stachowicz 2007), except for the sea urchin *A. lixula*, found on a panel. Other benthonic predators could have also interacted with panels during night or in periods different from those filmed, and their contribution to predation should be assessed in future experiments. Moreover, the cascading effects of large predators on meso-predator abundances could therefore be assessed, in order to deeper understand the trophic web of this experimental system (Lavender et al. 2014).

As far as the experimental set up used, the control of the artifact (cages) revealed no effects of cages, except for the exclusion of macropredators. Regarding the results of the experiment, two investigated variables resulted significantly different among conditions. In particular, Shannon Index and cover of native species resulted higher in caged panels. Hypothetically, these results could be due to the

absence of predation, that could not act as regulators of community dynamics because they were excluded by caging (Lafferty & Suchanek 2016).

The effect of predation on other variables measured was not observed. There are two possible explanations of these results: I) predation does not actually affect the abundance of the fouling growing on the panels (“wet weight” and “total cover” variables), the species richness (“S (tot)”, “S (native species)”, “S (NIS)”), and the NIS cover; II) the number of replicates in this experiment was not enough to highlight a slight effect. It has been observed for instance by NIS/native ratio, which seems to highlight NIS dominance when predators are excluded, although without statistical significance.

On the other hand, a strong effect of predation was observed exploring individual responses of NIS, namely the two species *B. luctuosum* and *S. plicata*, although they show an opposite pattern. *B. luctuosum* was indeed more abundant in caged panels, while *S. plicata* was more abundant in not-caged panels. These responses can result from the exclusion of predators and from interspecific competition, but could also depend on the mean size of the two species. In particular, *B. luctuosum* resulted a NIS predated by fish species, while *S. plicata* is not strongly influenced by predation, probably due to its physical-chemical defences (Lindquist et al. 1992). On the other hand, *S. plicata* loses competition with other species when the assemblage is not exposed to predators. Therefore, *S. plicata* could prevail on other species when there is consumption by predators. An analogue result was observed by Gauff et al. (2022), that found a positive effect of predation on the congeneric *Styela clava* Herdman, 1881.

In summary, the calcareous species were dominant in panels exposed to predators (Dias et al. 2020). This is an expected result, because “hard” species (like the calcareous ones) use this toughness as an effective anti-predator measure. Moreover, the question if NIS colonization is effectively penalized by predation of native species is still open, although we observed an opposite effect in two NIS well established in the study area. The effect of predation was highlighted for some variables (H' and native species % cover). Moreover, an effect on the structure of fouling assemblages from different experimental conditions was observed, analysing the whole fouling assemblages through multivariate analyses. In particular, assemblages excluded from predation resulted separated from the others, although a high similarity was found among all assemblages. Moreover, the homogeneity of multivariate variances indicated that the samples dispersion did not influence the PERMANOVA results, which showed differences between caged and uncaged assemblages. This experiment can also be considered as a study useful in the future, in combination with other repeated experiments, to assess the consumption rates at different latitudes in the Mediterranean Sea, that is still completely

unknown. The strength of predation pressure in this basin should be compared with that shown in tropical areas and at higher latitudes, adding new data for the verification of hypothesis to explain the latitudinal diversity gradient. In fact the high species diversity in the Mediterranean compared to that of other temperate regions, might offer an interesting complement to evaluate the hypothesis of predation as a driver of diversity – the so called “biotic interactions hypothesis” by Rodemann & Brandl (2017), that is still an open question in the literature.

A more powerful experiment will provide more information on the observed NIS dominance when predators are excluded. Further studies in the Mediterranean Sea are urgently needed, in order to confirm or not our results.

## 6. Final remarks

This Ph.D. project focused on the application of a new methodology for monitoring fouling NIS in the Mediterranean ports and investigated the colonization success of NIS in fouling communities through two different manipulative experiments, testing the resistance of native fouling communities and the effect of predation on NIS.

The monitoring allowed us to implement for the first time in the Mediterranean Sea an international standard protocol developed by the SERC to assess fouling communities and detect NIS. This monitoring protocol appears a strong and effective tool to early detect fouling sessile NIS in the Mediterranean ports. By the repeated use of SERC protocol in five marinas and one pristine site in the Gulf of La Spezia (Ligurian Sea) along a three-year series of seasonal monitoring during the growth period of fouling communities, its main advantages were assessed: cost-effectiveness, ease of application and the capability to obtain quantitative results in a relatively short time. On the other hand, the effort for sampling and analysing the panels is considerable and should be based on the availability of adequate knowledge capabilities. Therefore, here it is proposed the application of this method in other localities of the Mediterranean region, together with a comparison to other fouling monitoring procedures. The inclusion of innovative tools, like genetic approaches, is therefore promoted, in order to get as much as possible information on the status of biological invasion in ports of the Mediterranean Sea. We also suggest that this standard method could be internationally adopted by EU countries as a common monitoring strategy for NIS, following the indications of the Marine Strategy Framework Directive (Olenin et al. 2010).

Starting from the background of community structure and temporal dynamics of fouling communities in urbanized coastal environments in the studied localities, this research project also encompass two innovative experiments, set up in order to elucidate two mechanisms underlying the observed patterns in fouling assemblages, namely testing the biotic resistance hypothesis and the hypothesis on the role of predation.

The transplant experiments explored the resistance capability of native fouling communities against NIS colonization and provided additional information suggesting that the biotic resistance hypothesis could - at least partially - explain the ecological processes in the developing of fouling macroinvertebrate communities in the marina environment. The experimental design applied in this thesis should be easily repeated in other areas of the Mediterranean Sea, to obtain results leading to a generalized theory, useful not only from an ecological point of view, but also to better manage the biological invasion in the Mediterranean. First of all, our results can help the policy makers to aim for the development of new MPAs and for a better management of the existing ones in the

Mediterranean region. This strategy could be useful not only for the conservation of the native species richness, but also as “barriers” against NIS introduction. Secondly, these results could help to increase the knowledge of possible methods based on naturalization of urbanized marine sectors (e.g. ports), through the design, construction and functioning of nature-based coastal structures.

Predation exclusion experiments, by caging fouling assemblages, was also investigated for the first time in a site in the Mediterranean Sea. The effect of predation in shaping the structure of fouling communities was assessed, finding significant differences in two community variables - i.e. Shannon Index  $H'$  and mean cover of native species - and highlighting the different responses by two NIS, important components of the fouling assemblages commonly established in Mediterranean marinas. Due to a relatively low number of replicates, further experiments following the same methodologies should be promoted in the Mediterranean Sea, in order to understand in a more general manner the capability of native predators in the contrast against NIS invasion.

This project has thus investigated, by means of standard, repeated and careful monitoring methods, robust and valuable information on invasion biology of some components of the fouling communities of urbanized coastal sites and has pioneered manipulative ecological experiments that can shed new light on some mechanisms underlying the process of invasion, that is still underway with unprecedented magnitude in the Mediterranean Sea. Many questions are still open and require further efforts to be properly understood, so we hope that the results presented in this thesis could pave the way for future research.

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## Supplementary materials

**Supplementary table 1 Aim 1: List of native and cryptogenic species, including unresolved taxa (i.e., taxa with a low taxonomic resolution, which cannot be classified by biogeographic status), found in the sites of the three-year monitoring program. I: 2018; II: 2019; III: 2020. NIS are indicated in bold; unresolved taxa are underlined. Asterisks indicate NIS found for the first time in the Gulf of La Spezia. Abbreviations of sites are shown in Figure 3.1.**

(sub)Phylum	Taxon	FE			GR			PV			SP			ST		
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
<b>Porifera</b>	<i>Clathrina clathrus</i> (Schmidt, 1864)															
	<b>*<i>Paraleucilla magna</i> Klautau, Monteiro &amp; Borojevic, 2004</b>															
	<i>Porifera</i> ind.															
	<i>Sycon</i> sp.															
<b>Cnidaria</b>	<i>Actinia</i> sp.															
	<i>Aglaophenia</i> sp.															
	<i>Anemonia sulcata</i> (Pennant, 1777)															
	<i>Bougainvillia</i> sp.															
	<i>Clytia</i> sp.															
	<i>Condylactis aurantiaca</i> (Delle Chiaje, 1825)															
	<i>Cordylophora</i> sp.															
	<i>Corydendrium</i> sp.															
	<i>Ectopleura crocea</i> (Agassiz, 1862)															
	<i>Eudendrium racemosum</i> (Cavolini, 1785)															
	<i>Kirchenpaueria halecioides</i> (Alder, 1859)															
	<i>Pennaria disticha</i> Goldfuss, 1820															
	<b>Annelida</b>	<b>*<i>Branchiomma</i> sp.</b>														
<b>*<i>Branchiomma luctuosum</i> (Grube, 1870)</b>																
<i>Hydroides dianthus</i> (Verrill, 1873)																
<b><i>Hydroides dirampha</i> Mörch, 1863</b>																
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>																
<i>Janua</i> sp.																
<i>Protula</i> sp.																
<i>Sabella spallanzanii</i> (Gmelin, 1791)																
<i>Sabellinae</i> ind.																
<i>Salmacina</i> sp.																
<i>Serpula</i> sp.																
<i>Simplaria</i> sp.																
<i>Spirobranchus lamarcki</i> (Quatrefages, 1866)																
<i>Spirobranchus triqueter</i> (Linnaeus, 1758)																
<b>Mollusca</b>	<i>Anomia ephippium</i> Linnaeus, 1758															
	<i>Hiatella arctica</i> (Linnaeus, 1767)															
	<i>Lima lima</i> (Linnaeus, 1758)															
	<i>Mimachlamys varia</i> (Linnaeus, 1758)															
	<i>Musculus costulatus</i> (Risso, 1826)															
	<i>Mytilaster solidus</i> Monterosato, 1883															
	<i>Mytilus galloprovincialis</i> Lamarck, 1819															
<i>Ostrea edulis</i> Linnaeus, 1758																
<b>Crustacea</b>	<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)															
	<i>Perforatus perforatus</i> (Bruguère, 1789)															
<b>Bryozoa</b>	<i>Aetea</i> sp.															



**Supplementary table 2 Aim 2: List of taxa found in both study areas. NIS are shown in bold. Cryptogenic species are underlined. C: control panels; M: marina panels; T: transplanted panels.**

		<i>Leghorn</i>			<i>Gulf of La Spezia</i>		
		C	M	T	C	M	T
<b>Porifera</b>	<i>Clathrina</i> sp.		X		X		X
	<b><i>Paraleucilla magna</i> Klautau, Monteiro &amp; Borojevic, 2004</b>		X			X	X
	Porifera ind.			X	X	X	X
	<i>Sycon</i> sp.		X	X	X	X	X
<b>Cnidaria</b>	<i>Anemonia sulcata</i> (Pennant, 1777)	X				X	X
	<i>Aglaophenia</i> sp.				X		
	<i>Bougainvillia</i> sp.				X	X	X
	<i>Clytia</i> sp.				X	X	X
	<i>Eudendrium</i> sp.			X	X	X	X
	Hydrozoa ind.			X			X
	<i>Kirchenpaueria halecioides</i> (Alder, 1859)	X	X	X		X	
	<i>Pennaria disticha</i> Goldfuss, 1820					X	X
	<i>Sertularella</i> sp.		X	X			
	<b>Annelida</b>	<i>Bispira melanostigma</i> (Schmarda, 1861)					X
<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>						X	X
<b><i>Branchiomma</i> sp. (see Tamburini et al., 2021)</b>				X	X	X	X
<b><i>Ficopomatus enigmaticus</i> (Fauvel, 1923)</b>			X				
<i>Hydroides dianthus</i> (Verrill, 1873)			X			X	
<b><i>Hydroides dirampha</i> Mörch, 1863</b>			X			X	X
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>			X	X	X	X	X
<i>Janua</i> sp.		X	X	X	X	X	X
Sabellidae ind. 1					X		X
Sabellidae ind. 2					X	X	X
<i>Salmacina</i> sp.			X	X	X	X	X
Serpulidae ind. 1		X	X	X		X	X
<i>Simplaria</i> sp.		X	X	X	X	X	X
<i>Spirobranchus triqueter</i> (Linnaeus, 1758)		X		X			
<b>Mollusca</b>	<i>Anomia ephippium</i> Linnaeus, 1758	X	X	X	X	X	X
	<b><i>Arcuatula senhousia</i> (Benson, 1842)</b>				X		X
	<i>Lima lima</i> (Linnaeus, 1758)					X	
	<i>Mimachlamys varia</i> (Linnaeus, 1758)					X	X
	<i>Musculus costulatus</i> (Risso, 1826)				X		
	<i>Mytilaster solidus</i> Monterosato, 1883				X		
	<i>Mytilaster</i> sp. 1					X	
	<i>Mytilus galloprovincialis</i> Lamarck, 1819				X	X	X
	<i>Ostrea edulis</i> Linnaeus, 1758	X	X	X	X		X
<b>Crustacea</b>	<b><u><i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)</u></b>	X	X	X	X	X	X
	<i>Perforatus perforatus</i> (Bruguière, 1789)				X	X	X
<b>Entoprocta</b>	<i>Barentsia</i> sp.		X				
	<i>Pedicellina nutans</i> Dalyell, 1848				X		
<b>Bryozoa</b>	<i>Aetea</i> sp.		X		X	X	X
	<b><u><i>Amathia gracilis</i> (Leidy, 1855)</u></b>				X	X	
	<b><i>Amathia verticillata</i> (Delle Chiaje, 1822)</b>		X			X	



	<i>Bugula neritina</i> (Linnaeus, 1758)	X	X	X	X	X
	<i>Bugulina fulva</i> (Ryland, 1960)				X	
	<i>Bugulina stolonifera</i> (Ryland, 1960)	X	X		X	
	<b><i>Celleporaria brunnea</i> (Hincks, 1884)</b>		X		X	X
	<i>Conopeum seurati</i> (Canu, 1928)				X	
	<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)			X	X	X
	<i>Cradoscrupocellaria reptans</i> (Linnaeus, 1758)				X	
	<i>Crisia</i> sp.	X	X	X	X	X
	<i>Cryptosula pallasiana</i> (Moll, 1803)	X	X	X	X	X
	<i>Filicrisia</i> sp.			X		X
	<i>Savignyella lafontii</i> (Audouin, 1826)			X	X	X
	<i>Schizobrachiella sanguinea</i> (Norman, 1868)			X		X
	<i>Schizomavella</i> sp.			X		
	<i>Schizoporella errata</i> (Waters, 1878)			X	X	X
	<i>Schizoporella pungens</i> Canu & Bassler, 1928					X
	<b><i>Tricellaria inopinata</i> d'Hondt &amp; Occhipinti Ambrogi, 1985</b>		X	X	X	X
	<i>Turbicellepora magnicostata</i> (Barroso, 1919)			X		X
	<i>Umbonula oviceolata</i> Hastings, 1944					X
	<b><i>Watersipora arcuata</i> Banta, 1969</b>			X	X	
	<i>Watersipora complanata</i> (Norman, 1864)					X
	<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	X	X	X	X	X
<b>Tunicata</b>	Asciidiidae ind.	X	X		X	X
	<b><i>Botrylloides cf. niger</i> Herdman, 1886</b>	X	X			
	<i>Botryllus schlosseri</i> (Pallas, 1766)	X	X	X	X	X
	<i>Ciona</i> sp.				X	
	<i>Clavelina</i> sp.	X				
	Didemnidae ind.	X	X	X	X	X
	<i>Perophora</i> sp.	X	X		X	
	Stolidobranchia ind.	X	X	X	X	X
	<b><i>Styela plicata</i> (Lesueur, 1823)</b>	X		X	X	X

**Supplementary table 3 Aim 2: SIMPER table showing taxa contribution to similarity within condition in the Gulf of la Spezia. Data were log-transformed, and Bray-Curtis index was used to calculate similarity. Av.Abund: average abundance; Av.Sim: average similarity; Contrib%: single taxon contribution; Cum.%: cumulative percentage of contributions. NIS are shown in bold. C: control panels; M: marina panels; T: transplanted panels.**

<i>Group C (Average similarity: 76.84)</i>					
	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Salmacina</i> sp.	0.46	53.95	9.49	70.21	70.21
<i>Simplaria</i> sp.	0.13	13.33	3.49	17.34	87.56
<i>Schizoporella errata</i> (Waters, 1878)	0.01	1.45	2.97	1.89	89.45
<i>Crisia</i> sp.	0.01	1.25	34.48	1.63	91.07
<i>Group M (Average similarity: 33.83)</i>					
	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Anomia ephippium</i> Linnaeus, 1758	0.11	6.93	0.76	20.49	20.49
<i>Crisia</i> sp.	0.12	5.71	0.71	16.87	37.36
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.05	3.69	1.1	10.92	48.28
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.13	3.2	0.43	9.45	57.73
<i>Simplaria</i> sp.	0.04	2.43	1.16	7.17	64.9
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.03	1.99	1.05	5.87	70.77
<i>Schizoporella errata</i> (Waters, 1878)	0.05	1.78	0.54	5.26	76.03
<b><i>Celleporaria brunnea</i> (Hincks, 1884)</b>	0.02	1.18	1.6	3.49	79.53
Stolidobranchia ind.	0.01	0.93	1.08	2.75	82.28
<i>Sycon</i> sp.	0.01	0.85	1.24	2.53	84.8
Asciidiidae ind.	0.03	0.85	0.7	2.51	87.31
Sabellinae ind.	0.01	0.77	1.25	2.28	89.59
<i>Bugula neritina</i> (Linnaeus, 1758)	0.01	0.59	0.91	1.74	91.33
<i>Group T (Average similarity: 37.57)</i>					
	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Salmacina</i> sp.	0.15	8.4	0.73	22.36	22.36
<i>Schizoporella errata</i> (Waters, 1878)	0.1	6.42	1.2	17.07	39.44
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.11	4.73	0.71	12.59	52.02
<i>Crisia</i> sp.	0.13	4.52	0.54	12.03	64.05
<i>Simplaria</i> sp.	0.09	3.8	0.99	10.12	74.17
<i>Anomia ephippium</i> Linnaeus, 1758	0.02	1.36	3.65	3.62	77.78
Stolidobranchia ind.	0.02	1.35	3.93	3.61	81.39
<i>Perforatus perforatus</i> (Bruguière, 1789)	0.01	1.27	9.94	3.38	84.77
<i>Sycon</i> sp.	0.01	0.83	1.35	2.2	86.98
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.01	0.81	1.36	2.15	89.13
Asciidiidae ind.	0.01	0.81	1.36	2.15	91.28

Supplementary table 4 Aim 2: SIMPER table showing taxa contribution to dissimilarity between conditions in the Gulf of La Spezia. Data were log-transformed, and Bray-Curtis index was used to calculate dissimilarity. Av.Abund: average abundance; Av.Diss: average dissimilarity; Contrib%: single taxon contribution; Cum.%: cumulative percentage of contributions. NIS are shown in bold. C: control panels; M: marina panels; T: transplanted panels.

	Group C		Group M			
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<b>Average dissimilarity: 85.22</b>						
<i>Salmacina</i> sp.	0.46	0.01	29.3	6.17	34.38	34.38
<i>Crisia</i> sp.	0.01	0.12	7.19	0.78	8.43	42.81
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.01	0.13	7.17	0.69	8.41	51.23
<i>Anomia ephippium</i> Linnaeus, 1758	0.01	0.11	6.86	0.97	8.06	59.28
<i>Simplaria</i> sp.	0.13	0.04	5.95	2.11	6.98	66.26
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0	0.05	3.35	1.45	3.93	70.19
<i>Schizoporella errata</i> (Waters, 1878)	0.01	0.05	2.72	0.79	3.19	73.38
<i>Janua</i> sp.	0.03	0	2.16	0.62	2.53	75.91
Asciidiidae ind.	0	0.03	1.88	0.58	2.21	78.12
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.01	0.03	1.63	0.76	1.91	80.03
<b><i>Celleporaria brunnea</i> (Hincks, 1884)</b>	0	0.02	1.07	1.24	1.26	81.29
<b><i>Hydroides dirampha</i> Mörch, 1863</b>	0	0.02	1	0.49	1.18	82.47
<i>Botryllus schlosseri</i> (Pallas, 1766)	0	0.02	0.96	0.6	1.13	83.6
<i>Ostrea edulis</i> Linnaeus, 1758	0.01	0	0.91	0.57	1.07	84.67
<i>Cradoscrupocellaria reptans</i> (Linnaeus, 1758)	0	0.01	0.91	0.36	1.07	85.74
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0	0.01	0.81	0.43	0.95	86.69
<i>Perforatus perforatus</i> (Bruguère, 1789)	0.01	0	0.77	1.43	0.9	87.59
<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>	0	0.01	0.72	0.73	0.85	88.44
<i>Savignyella lafontii</i> (Audouin, 1826)	0	0.01	0.72	0.85	0.84	89.28
Sabellinae ind.	0	0.01	0.58	0.96	0.68	89.96
<i>Mytilus galloprovincialis</i> Lamarck, 1819	0	0.01	0.56	0.42	0.66	90.62
	Group C		Group T			
<b>Average dissimilarity: 61.01</b>						
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>Salmacina</i> sp.	0.46	0.15	19.38	2.17	31.76	31.76
<i>Crisia</i> sp.	0.01	0.13	7.1	0.79	11.64	43.4
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.01	0.11	6.48	0.97	10.62	54.02
<i>Simplaria</i> sp.	0.13	0.09	6.38	1.8	10.46	64.49
<i>Schizoporella errata</i> (Waters, 1878)	0.01	0.1	5.56	1.1	9.12	73.61
<i>Janua</i> sp.	0.03	0	2.08	0.59	3.41	77.02
<b><i>Celleporaria brunnea</i> (Hincks, 1884)</b>	0	0.02	1.33	0.69	2.18	79.19
<i>Ostrea edulis</i> Linnaeus, 1758	0.01	0.01	1.21	0.83	1.98	81.17
<i>Anomia ephippium</i> Linnaeus, 1758	0.01	0.02	0.87	0.71	1.43	82.6

<i>Bugula neritina</i> (Linnaeus, 1758)	0	0.01	0.84	0.72	1.37	83.98
<i>Botryllus schlosseri</i> (Pallas, 1766)	0	0.01	0.82	0.73	1.35	85.33
Asciidiidae ind.	0	0.01	0.61	1.8	0.99	86.32
<i>Clathrina</i> sp.	0.01	0	0.48	1.06	0.78	87.11
<i>Perforatus perforatus</i> (Bruguère, 1789)	0.01	0.01	0.47	1.39	0.77	87.87
<i>Pennaria disticha</i> Goldfuss, 1820	0	0.01	0.43	0.87	0.71	88.58
Stolidobranchia ind.	0.01	0.02	0.42	0.58	0.69	89.26
Didemnidae ind.	0	0	0.32	0.97	0.53	89.79
<i>Bougainvillia</i> sp.	0	0	0.32	0.97	0.52	90.31

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**Group M    Group T**

**Average dissimilarity: 71.04**

	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>Salmacina</i> sp.	0.01	0.15	9.97	0.99	14.04	14.04
<i>Crisia</i> sp.	0.12	0.13	9.85	1.12	13.86	27.9
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.13	0.11	9.65	1.07	13.58	41.48
<i>Anomia ephippium</i> Linnaeus, 1758	0.11	0.02	6.6	0.97	9.29	50.77
<i>Schizoporella errata</i> (Waters, 1878)	0.05	0.1	5.69	1.18	8.01	58.79
<i>Simplaria</i> sp.	0.04	0.09	5.06	0.87	7.12	65.91
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.05	0	3.15	1.38	4.43	70.34
Asciidiidae ind.	0.03	0.01	1.75	0.57	2.47	72.8
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.03	0.01	1.6	0.74	2.25	75.06
<b><i>Celleporaria brunnea</i> (Hincks, 1884)</b>	0.02	0.02	1.45	0.87	2.04	77.1
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.02	0.01	1.32	0.81	1.86	78.96
<b><i>Hydroides dirampha</i> Mörch, 1863</b>	0.02	0	1.15	0.59	1.62	80.58
<i>Cradoscrupocellaria reptans</i> (Linnaeus, 1758)	0.01	0	0.92	0.36	1.29	81.87
<i>Bugula neritina</i> (Linnaeus, 1758)	0.01	0.01	0.88	0.79	1.24	83.11
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0.01	0	0.82	0.43	1.15	84.27
<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>	0.01	0	0.77	0.81	1.09	85.36
Stolidobranchia ind.	0.01	0.02	0.73	0.99	1.03	86.39
<i>Savignyella lafontii</i> (Audouin, 1826)	0.01	0	0.73	0.85	1.02	87.41
<i>Perforatus perforatus</i> (Bruguère, 1789)	0	0.01	0.69	2.06	0.97	88.39
<i>Mytilus galloprovincialis</i> Lamarck, 1819	0.01	0	0.61	0.46	0.86	89.25
<i>Ostrea edulis</i> Linnaeus, 1758	0	0.01	0.61	0.64	0.85	90.1

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**Supplementary table 5 Aim 2: SIMPER table showing taxa contribution to similarity within condition in the Leghorn area. Data were log-transformed, and Bray-Curtis index was used to calculate similarity. Av.Abund: average abundance; Av.Sim: average similarity; Contrib%: single taxon contribution; Cum.%: cumulative percentage of contributions. NIS are shown in bold. C: control panels; M: marina panels; T: transplanted panels.**

<i>Group C (Average similarity: 69.08)</i>					
	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Janua</i> sp.	0.05	44.48	3.63	64.39	64.39
<i>Simplaria</i> sp.	0.01	12.87	4.6	18.64	83.03
<i>Stolidobranchia</i> ind.	0.01	8.1	1.3	11.72	94.75
<i>Group M (Average similarity: 46.83)</i>					
	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.17	12.39	0.61	26.45	26.45
<i>Salmacina</i> sp.	0.11	8.63	0.53	18.42	44.88
<i>Janua</i> sp.	0.02	4.01	24.64	8.56	53.44
Ascidiidae ind.	0.01	2.36	2.65	5.04	58.48
<b><i>Botrylloides cf. niger</i> Herdman, 1886</b>	0.03	2.36	2.67	5.04	63.52
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.01	2.01	24.64	4.3	67.82
<i>Simplaria</i> sp.	0.01	2.01	24.64	4.3	72.12
Didemnidae ind.	0.01	2.01	24.64	4.3	76.43
<i>Stolidobranchia</i> ind.	0.01	2.01	24.64	4.3	80.73
<i>Bugulina stolonifera</i> (Ryland, 1960)	0.01	1.04	0.91	2.23	82.96
<b><i>Amathia verticillata</i> (Delle Chiaje, 1822)</b>	0.01	1	0.91	2.13	85.09
<i>Crisia</i> sp.	0.01	1	0.91	2.13	87.22
<i>Sycon</i> sp.	0.01	1	0.91	2.13	89.35
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.01	0.99	0.91	2.12	91.47
<i>Group T (Average similarity: 50.77)</i>					
	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Janua</i> sp.	0.08	11.7	1.1	23.04	23.04
<b><i>Botrylloides cf. niger</i> Herdman, 1886</b>	0.02	5.4	2.23	10.64	33.68
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.04	5.33	2.49	10.49	44.17
<i>Salmacina</i> sp.	0.01	3.84	4.29	7.56	51.73
Didemnidae ind.	0.01	3.84	4.29	7.56	59.3
<i>Stolidobranchia</i> ind.	0.01	3.84	4.29	7.56	66.86
<i>Simplaria</i> sp.	0.01	2.77	1.29	5.45	72.31
<i>Cryptosula pallasiana</i> (Moll, 1803)	0.01	2.7	1.27	5.31	77.62
<i>Ostrea edulis</i> Linnaeus, 1758	0.01	2.42	1.28	4.76	82.38
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.01	2.17	0.74	4.28	86.66
<i>Bugulina stolonifera</i> (Ryland, 1960)	0.01	1.52	0.76	3	89.66
<i>Sycon</i> sp.	0.01	1.5	0.77	2.96	92.62

**Supplementary table 6 Aim 2: SIMPER table showing taxa contribution to dissimilarity between conditions in the Leghorn area. Data were log-transformed, and Bray-Curtis index was used to calculate dissimilarity. Av.Abund: average abundance; Av.Diss: average dissimilarity; Contrib%: single taxon contribution; Cum.%: cumulative percentage of contributions. NIS are shown in bold. C: control panels; M: marina panels; T: transplanted panels.**

	Group C		Group M			
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<b>Average dissimilarity: 84.06</b>						
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0	0.17	28.21	1.11	33.56	33.56
<i>Salmacina</i> sp.	0	0.11	20.18	1.06	24.01	57.57
<b><i>Botrylloides cf. niger</i> Herdman, 1886</b>	0	0.03	6.04	0.91	7.19	64.76
<i>Janua</i> sp.	0.05	0.02	4.41	1.77	5.24	70
Asciidiidae ind.	0	0.01	2.63	2.69	3.13	73.13
<i>Botryllus schlosseri</i> (Pallas, 1766)	0	0.01	2.13	3.5	2.53	75.67
Didemnidae ind.	0	0.01	1.74	14.55	2.07	77.73
Serpulidae ind.	0	0.01	1.68	0.97	2	79.74
<i>Bugulina stolonifera</i> (Ryland, 1960)	0	0.01	1.34	1.69	1.6	81.33
<b><i>Amathia verticillata</i> (Delle Chiaje, 1822)</b>	0	0.01	1.29	1.68	1.54	82.87
<i>Crisia</i> sp.	0	0.01	1.29	1.68	1.54	84.41
<i>Sycon</i> sp.	0	0.01	1.29	1.68	1.54	85.95
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0	0.01	1.29	1.68	1.53	87.48
<i>Clavelina</i> sp.	0	0.01	1.29	1.68	1.53	89.02
<i>Hydroides dianthus</i> (Verrill, 1873)	0	0	0.89	0.98	1.06	90.08
	Group C		Group T			
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<b>Average dissimilarity: 66.25</b>						
<i>Janua</i> sp.	0.05	0.08	15.04	1.26	22.7	22.7
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0	0.04	9.25	0.96	13.96	36.66
<b><i>Botrylloides cf. niger</i> Herdman, 1886</b>	0	0.02	5.1	2.35	7.7	44.36
<i>Botryllus schlosseri</i> (Pallas, 1766)	0	0.01	4.09	1.19	6.17	50.53
Asciidiidae ind.	0	0.01	3.52	0.76	5.31	55.84
<i>Salmacina</i> sp.	0	0.01	3.4	3.96	5.13	60.97
Didemnidae ind.	0	0.01	3.04	3.6	4.59	65.56
<i>Cryptosula pallasiana</i> (Moll, 1803)	0	0.01	2.29	1.41	3.46	69.02
<i>Ostrea edulis</i> Linnaeus, 1758	0	0.01	2.24	0.89	3.38	72.4
<i>Bugulina stolonifera</i> (Ryland, 1960)	0	0.01	2.04	1.24	3.08	75.48
<i>Sycon</i> sp.	0	0.01	1.98	1.3	2.99	78.47
<i>Perophora</i> sp.	0	0	1.9	0.97	2.87	81.33
Serpulidae ind.	0	0.01	1.7	0.93	2.56	83.9
<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)	0	0	1.46	0.93	2.21	86.1
<i>Anomia ephippium</i> Linnaeus, 1758	0	0	1.33	0.84	2	88.1
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0	0	1.06	0.65	1.6	89.71

<i>Spirobranchus triqueter</i> (Linnaeus, 1758)	0	0	0.96	0.58	1.45	91.16
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**Group M    Group T**

**Average dissimilarity: 62.39**

	<b>Av.Abund</b>	<b>Av.Abund</b>	<b>Av.Diss</b>	<b>Diss/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.17	0.04	19.42	1.05	31.13	31.13
<i>Salmacina</i> sp.	0.11	0.01	13.9	0.97	22.28	53.41
<i>Janua</i> sp.	0.02	0.08	7.51	0.96	12.04	65.44
<b><i>Botrylloides cf. niger</i> Herdman, 1886</b>	0.03	0.02	3.22	0.71	5.16	70.61
Asciidiidae ind.	0.01	0.01	2.08	1.28	3.34	73.95
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.01	0.01	1.46	1.36	2.35	76.29
Serpulidae ind.	0.01	0.01	1.3	1.18	2.08	78.38
<b><i>Amathia verticillata</i> (Delle Chiaje, 1822)</b>	0.01	0	0.98	1.65	1.58	79.95
<i>Clavelina</i> sp.	0.01	0	0.98	1.65	1.57	81.52
<i>Cryptosula pallasiana</i> (Moll, 1803)	0	0.01	0.91	1.35	1.45	82.97
<i>Crisia</i> sp.	0.01	0	0.86	1.36	1.38	84.35
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.01	0	0.76	1.14	1.22	85.58
<i>Ostrea edulis</i> Linnaeus, 1758	0.01	0.01	0.71	0.82	1.13	86.71
<i>Hydroides dianthus</i> (Verrill, 1873)	0	0	0.67	0.97	1.08	87.79
<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)	0	0	0.66	0.96	1.06	88.85
<i>Perophora</i> sp.	0	0	0.66	0.97	1.05	89.9
<i>Anomia ephippium</i> Linnaeus, 1758	0	0	0.65	0.96	1.05	90.95

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**Supplementary table 7 Aim 3: List of taxa found in the panels of predation exclusion experiment. NIS are in bold type. C: control panels; Ca: caged panels; HCa: half-caged panels.**

		C	Ca	HCa
Porifera	<b><i>Paraleucilla magna</i> Klautau, Monteiro &amp; Borojevic, 2004</b>			
	Porifera ind.			
Cnidaria	<i>Actinia</i> sp.			
	<i>Clytia</i> sp.			
	<i>Ectopleura</i> sp.			
	<i>Eudendrium</i> sp.			
	<i>Pennaria disticha</i> Goldfuss, 1820			
Annelida	<b><i>Branchiomma</i> sp. (see Tamburini et al. 2021)</b>			
	<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>			
	<i>Hydroides dianthus</i> (Verrill, 1873)			
	<b><i>Hydroides dirampha</i> Mörch, 1863</b>			
	<b><i>Hydroides elegans</i> (Haswell, 1883)</b>			
	<i>Janua</i> sp.			
	<i>Simplaria</i> sp.			
	<i>Sabella</i> sp.			
	<i>Salmacina</i> sp.			
	<i>Spirobranchus</i> sp.			
	<i>Spirobranchus tetraceros</i> (Schmarda, 1861)			
Mollusca	<i>Anomia ephippium</i> Linnaeus, 1758			
	<i>Ostrea edulis</i> Linnaeus, 1758			
Crustacea	<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)			
	<i>Amphibalanus eburneus</i> (Gould, 1841)			
	<i>Perforatus perforatus</i> (Bruguère, 1789)			
	<i>Aetea</i> sp.			
Bryozoa	<b><i>Amathia verticillata</i> (Delle Chiaje, 1822)</b>			
	<i>Bugula neritina</i> (Linnaeus, 1758)			
	<i>Bugulina fulva</i> (Ryland, 1960)			
	<i>Bugulina stolonifera</i> (Ryland, 1960)			
	<b><i>Celleporaria brunnea</i> (Hincks, 1884)</b>			
	<i>Conopeum seurati</i> (Canu, 1928)			
	<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)			
	<i>Cradoscrupocellaria reptans</i> (Linnaeus, 1758)			
	<i>Crisia denticulata</i> (Lamarck, 1816)			
	<i>Crisia eburnea</i> (Linnaeus, 1758)			
	<i>Schizoporella errata</i> (Waters, 1878)			
	<b><i>Tricellaria inopinata</i> d'Hondt &amp; Occhipinti Ambrogi, 1985</b>			
	<i>Watersipora subtorquata</i> (d'Orbigny, 1852)			
Tunicata	<i>Ascidia</i> sp.			
	<i>Botryllus schlosseri</i> (Pallas, 1766)			
	Didemnidae ind.			
	<i>Diplosoma</i> sp.			
	<i>Perophora</i> sp.			
	<i>Phallusia mammillata</i> (Cuvier, 1815)			
	Stolidobranchia ind.			
	<b><i>Styela plicata</i> (Lesueur, 1823)</b>			



Supplementary table 8 Aim 3: SIMPER table showing taxa contribution to dissimilarity within conditions in the experiment. Data were square-root transformed, and Bray-Curtis index was used to calculate dissimilarity.

Av.Abund: average abundance; Av.Diss: average dissimilarity; Sim/SD: ratio between similarity and standard deviation. Contrib%: single taxon contribution; Cum.%: cumulative percentage of contributions. NIS are shown in bold. C: control panels; Ca: caged panels; HCa: half-caged panels.

<b>Group C</b>					
<b>Average similarity: 71.57</b>					
<b>Species</b>	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Schizoporella errata</i> (Waters, 1878)	0.8	17.43	11.05	24.36	24.36
<i>Salmacina</i> sp.	0.53	9.53	3.38	13.32	37.68
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.37	7.13	5.01	9.96	47.63
<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)	0.25	4.62	3.8	6.46	54.09
<i>Anomia ephippium</i> Linnaeus, 1758	0.18	3.49	3.07	4.88	58.97
<i>Bugulina fulva</i> (Ryland, 1960)	0.19	3.45	4.57	4.82	63.79
<b><i>Branchiomma</i> sp. (see Tamburini et al. 2021)</b>	0.14	2.49	5.27	3.48	67.27
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.13	2.49	5.27	3.48	70.76
<i>Crisia eburnea</i> (Linnaeus, 1758)	0.12	2.48	6.84	3.46	74.22
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.14	2.48	6.84	3.46	77.68
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.14	1.85	1.24	2.59	80.28
<i>Perophora</i> sp.	0.14	1.8	1.26	2.51	82.79
<i>Sabella</i> sp.	0.11	1.72	1.31	2.4	85.18
<i>Clytia</i> sp.	0.11	1.63	1.34	2.28	87.46
<i>Hydroides dianthus</i> (Verrill, 1873)	0.11	1.58	1.35	2.21	89.67
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0.09	1.53	1.36	2.14	91.81

<b>Group Ca</b>					
<b>Average similarity: 63.08</b>					
<b>Species</b>	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Schizoporella errata</i> (Waters, 1878)	0.43	7.48	3.46	11.87	11.87
<i>Anomia ephippium</i> Linnaeus, 1758	0.37	5.93	1.99	9.4	21.27
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.3	4.82	2.74	7.64	28.91
<i>Salmacina</i> sp.	0.34	4.7	1.28	7.45	36.36
<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>	0.25	4.27	3.31	6.78	43.14
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.25	3.58	1.93	5.68	48.82
<i>Sabella</i> sp.	0.19	3.09	3.16	4.91	53.72
<b><i>Branchiomma</i> sp. (see Tamburini et al. 2021)</b>	0.16	2.87	4.1	4.54	58.27
Didemnidae ind.	0.21	2.77	3.36	4.39	62.66
<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)	0.15	2.62	3.85	4.15	66.81
<i>Crisia eburnea</i> (Linnaeus, 1758)	0.18	2.46	2.28	3.9	70.71
<i>Janua</i> sp.	0.12	2.34	6.72	3.72	74.43
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.16	2.23	9.48	3.53	77.96
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0.14	1.9	1.13	3.02	80.98
<i>Ascidia</i> sp.	0.2	1.74	0.65	2.76	83.73
<i>Clytia</i> sp.	0.16	1.69	1.28	2.68	86.42
<i>Simplaria</i> sp.	0.1	1.55	1.34	2.46	88.88
<i>Perophora</i> sp.	0.12	1.52	1.26	2.41	91.29

<b>Group HCa</b>					
<b>Average similarity: 66.75</b>					
<b>Species</b>	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>

<i>Schizoporella errata</i> (Waters, 1878)	0.72	13.24	1.44	19.83	19.83
<i>Salmacina</i> sp.	0.4	6.72	2.48	10.07	29.9
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.27	5.13	3.35	7.68	37.58
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.29	4.95	4.34	7.41	45
<i>Anomia ephippium</i> Linnaeus, 1758	0.25	3.93	3.34	5.89	50.89
<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)	0.17	3	4.07	4.49	55.38
<b><i>Branchiomma</i> sp. (see Tamburini et al. 2021)</b>	0.17	2.96	3.21	4.43	59.81
<i>Bugulina fulva</i> (Ryland, 1960)	0.16	2.9	4.27	4.34	64.15
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.15	2.84	5.5	4.26	68.41
<i>Sabella</i> sp.	0.13	2.62	5.67	3.92	72.33
<i>Bugula neritina</i> (Linnaeus, 1758)	0.11	2.35	7.95	3.53	75.86
<i>Crisia eburnea</i> (Linnaeus, 1758)	0.12	2.06	1.46	3.09	78.95
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0.1	1.81	1.52	2.72	81.67
<i>Janua</i> sp.	0.11	1.79	1.48	2.68	84.34
<i>Clytia</i> sp.	0.11	1.76	1.51	2.64	86.99
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.11	1.67	1.5	2.5	89.48
<b><i>Paraleucilla magna</i> Klautau, Monteiro &amp; Borojevic, 2004</b>	0.07	1.15	0.92	1.72	91.2

**Supplementary table 9 Aim 3: SIMPER table showing taxa contribution to dissimilarity between conditions in the experiment of aim 3. Data were square-root transformed, and Bray-Curtis index was used to calculate dissimilarity. Av.Abund: average abundance; Av.Diss: average dissimilarity; Diss/SD: ratio between dissimilarity and standard deviation. Contrib%: single taxon contribution; Cum.%: cumulative percentage of contributions. NIS are shown in bold. C: control panels; Ca: caged panels; HCa: half-caged panels.**

#### Groups C & Ca

Average dissimilarity =  
42.28

Species	Group C		Group Ca		Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund	Av.Diss	Av.Diss			
<i>Schizoporella errata</i> (Waters, 1878)	0.8	0.43	4.1	2.52	9.7	9.7	
<i>Salmacina</i> sp.	0.53	0.34	2.86	1.33	6.76	16.46	
<i>Anomia ephippium</i> Linnaeus, 1758	0.18	0.37	2.44	1.88	5.77	22.24	
<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>	0.04	0.25	2.37	2.01	5.61	27.84	
<i>Ascidia</i> sp.	0.05	0.2	1.96	1.26	4.64	32.49	
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.14	0.3	1.85	1.58	4.37	36.85	
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.37	0.25	1.74	1.37	4.1	40.96	
Didemnidae ind.	0.05	0.21	1.73	1.08	4.09	45.05	
<i>Bugulina fulva</i> (Ryland, 1960)	0.19	0.06	1.68	1.6	3.97	49.02	
<i>Crisia denticulata</i> (Lamarck, 1816)	0.11	0.11	1.66	1.1	3.93	52.95	
<i>Diplosoma</i> sp.	0.1	0.04	1.35	0.68	3.2	56.15	
<i>Clytia</i> sp.	0.11	0.16	1.22	0.97	2.88	59.02	
<i>Amphibalanus amphitrite</i> <i>amphitrite</i> (Darwin, 1854)	0.25	0.15	1.18	1.37	2.78	61.81	

<i>Botryllus schlosseri</i> (Pallas, 1766)	0.14	0.16	1.14	1.03	2.69	64.5
<i>Simplaria</i> sp.	0	0.1	1.1	2.05	2.6	67.1
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.13	0.04	1.07	1.64	2.53	69.63
<i>Hydroides dianthus</i> (Verrill, 1873)	0.11	0.02	1.06	1.54	2.51	72.15
<i>Perophora</i> sp.	0.14	0.12	1.06	1.14	2.5	74.65
<i>Sabella</i> sp.	0.11	0.19	0.98	1.2	2.33	76.97
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0.09	0.14	0.97	1.31	2.29	79.26
<i>Crisia eburnea</i> (Linnaeus, 1758)	0.12	0.18	0.91	0.91	2.14	81.4
<b><i>Paraleucilla magna</i> Klautau, Monteiro &amp; Borojevic, 2004</b>	0.03	0.09	0.76	1.3	1.8	83.2
<i>Amphibalanus eburneus</i> (Gould, 1841)	0.07	0.03	0.7	1.14	1.65	84.85
<b><i>Branchiomma</i> sp. (see Tamburini et al. 2021)</b>	0.14	0.16	0.64	1.1	1.51	86.37
<b><i>Hydroides dirampha</i> Mörch, 1863</b>	0.02	0.06	0.63	0.99	1.49	87.86
<i>Bugula neritina</i> (Linnaeus, 1758)	0.07	0.1	0.61	0.93	1.44	89.29
<i>Eudendrium</i> sp.	0.05	0	0.57	0.98	1.34	90.64

**Groups C & HCa**  
Average dissimilarity = **30.79**

Species	Group		Av.Diss	Diss/SD	Contrib%	Cum.%
	Group C Av.Abund	HCa Av.Abund				
<i>Salmacina</i> sp.	0.53	0.4	2.59	1.34	8.43	8.43
<i>Schizoporella errata</i> (Waters, 1878)	0.8	0.72	2.36	0.79	7.65	16.08
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.14	0.29	1.76	1.44	5.71	21.79
<i>Crisia denticulata</i> (Lamarck, 1816)	0.11	0.09	1.55	1.01	5.02	26.81
<i>Diplosoma</i> sp.	0.1	0.05	1.52	0.72	4.93	31.74
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.37	0.27	1.41	1.45	4.58	36.32
<i>Anomia ephippium</i> Linnaeus, 1758	0.18	0.25	1.23	0.97	4	40.33
<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>	0.04	0.11	1.22	1.2	3.95	44.28
<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)	0.25	0.17	1.19	1.28	3.85	48.13
<i>Perophora</i> sp.	0.14	0.08	1.12	1.05	3.65	51.78
<i>Botryllus schlosseri</i> (Pallas, 1766)	0.14	0.11	1.01	1.16	3.29	55.07
<i>Hydroides dianthus</i> (Verrill, 1873)	0.11	0.04	0.9	1.23	2.94	58.01

<i>Bugulina fulva</i> (Ryland, 1960)	0.19	0.16	0.85	1.07	2.76	60.77
<i>Amphibalanus eburneus</i> (Gould, 1841)	0.07	0.06	0.83	1.2	2.71	63.48
Didemnidae ind.	0.05	0.08	0.8	1.01	2.6	66.08
<b><i>Branchiomma</i> sp. (see Tamburini et al. 2021)</b>	0.14	0.17	0.78	1.04	2.54	68.62
<i>Clytia</i> sp.	0.11	0.11	0.7	1.09	2.27	70.9
<b><i>Paraleucilla magna</i> Klautau, Monteiro &amp; Borojevic, 2004</b>	0.03	0.07	0.67	1.13	2.19	73.09
<i>Sabella</i> sp.	0.11	0.13	0.61	1.06	1.98	75.07
<i>Ascidia</i> sp.	0.05	0.06	0.58	0.98	1.89	76.96
<i>Eudendrium</i> sp.	0.05	0.06	0.58	0.98	1.89	78.85
Stolidobranchia ind.	0.03	0.04	0.55	0.93	1.77	80.62
<i>Janua</i> sp.	0.08	0.11	0.54	0.85	1.76	82.38
<i>Crisia eburnea</i> (Linnaeus, 1758)	0.12	0.12	0.53	0.95	1.73	84.11
<i>Simplaria</i> sp.	0	0.04	0.49	0.85	1.59	85.7
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.13	0.15	0.47	1.17	1.53	87.23
<i>Bugula neritina</i> (Linnaeus, 1758)	0.07	0.11	0.46	0.78	1.5	88.73
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0.09	0.1	0.46	0.88	1.48	90.21

**Groups Ca & HCa**  
**Average dissimilarity = 38.93**

Species	Group		Av.Diss	Diss/SD	Contrib%	Cum.%
	Group Ca Av.Abund	HCa Av.Abund				
<i>Schizoporella errata</i> (Waters, 1878)	0.43	0.72	4.72	2.44	12.13	12.13
<i>Anomia ephippium</i> Linnaeus, 1758	0.37	0.25	2.3	1.55	5.92	18.04
<i>Salmacina</i> sp.	0.34	0.4	2.21	1.29	5.69	23.73
<i>Ascidia</i> sp.	0.2	0.06	1.97	1.27	5.05	28.78
<b><i>Branchiomma luctuosum</i> (Grube, 1870)</b>	0.25	0.11	1.86	1.36	4.79	33.57
Didemnidae ind.	0.21	0.08	1.7	1.09	4.36	37.93
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	0.3	0.29	1.45	1.41	3.74	41.66
<i>Crisia denticulata</i> (Lamarck, 1816)	0.11	0.09	1.43	1.08	3.68	45.34
<b><i>Hydroides elegans</i> (Haswell, 1883)</b>	0.25	0.27	1.4	1.54	3.6	48.95
<i>Bugulina fulva</i> (Ryland, 1960)	0.06	0.16	1.39	1.78	3.58	52.53
<i>Clytia</i> sp.	0.16	0.11	1.22	0.95	3.13	55.65
<b><i>Styela plicata</i> (Lesueur, 1823)</b>	0.04	0.15	1.21	1.81	3.1	58.75
<i>Crisia eburnea</i> (Linnaeus, 1758)	0.18	0.12	1.17	1.21	3	61.75

<i>Botryllus schlosseri</i> (Pallas, 1766)	0.16	0.11	0.96	0.77	2.47	64.22
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	0.14	0.1	0.95	1.4	2.43	66.65
<i>Perophora</i> sp.	0.12	0.08	0.81	1.1	2.09	68.74
<i>Diplosoma</i> sp.	0.04	0.05	0.81	0.82	2.07	70.8
<i>Sabella</i> sp.	0.19	0.13	0.79	1.18	2.03	72.83
<i>Simplaria</i> sp.	0.1	0.04	0.78	1.23	2.01	74.84
<b><i>Branchiomma</i> sp. (see Tamburini et al. 2021)</b>	0.16	0.17	0.75	1.16	1.93	76.78
<i>Amphibalanus amphitrite amphitrite</i> (Darwin, 1854)	0.15	0.17	0.74	1.24	1.9	78.68
<i>Amphibalanus eburneus</i> (Gould, 1841)	0.03	0.06	0.69	0.92	1.78	80.46
<b><i>Hydroides dirampha</i> Mörch, 1863</b>	0.06	0.01	0.64	0.97	1.64	82.1
<i>Eudendrium</i> sp.	0	0.06	0.62	1.13	1.58	83.68
<b><i>Tricellaria inopinata</i> d'Hondt &amp; Occhipinti Ambrogi, 1985</b>	0.05	0.01	0.55	0.98	1.4	85.08
<i>Stolidobranchia</i> ind.	0.03	0.04	0.53	0.93	1.36	86.44
<i>Hydroides dianthus</i> (Verrill, 1873)	0.02	0.04	0.5	0.89	1.29	87.73
<b><i>Paraleucilla magna</i> Klautau, Monteiro &amp; Borojevic, 2004</b>	0.09	0.07	0.47	0.84	1.22	88.95
<i>Janua</i> sp.	0.12	0.11	0.47	1.03	1.21	90.17

## **Acknowledgments**

Condensing three years of experiences, encounters, efforts, achievements and failures in a few lines is certainly a hard challenge and it does not fully describe the beautiful adventure I played. Here I will try to adequately thank all the people involved in this Ph.D. project.

First of all, thanks to Prof. Anna Occhipinti and Dr Jasmine Ferrario for their invaluable support and for having pushed me to improve myself both as a researcher and as a person.

Thank you, Dr Agnese Marchini, for scientific and moral support, for the laughs and the conversations and for having believed in me. Thanks to Dr Daniele Paganelli, for his “male” help in a lab full of women.

I wish to thank Dr Gregory Ruiz, Dr Erika Keppel and Dr Michele Repetto of the Smithsonian Environmental Research Center (SERC) in Maryland (USA) for their teachings on the monitoring protocol and because they showed me how to be a good scientist. I hope I can meet them again in the future.

A special thanks to Dr Chiara Lombardi of ENEA, that provided us the labs where the monitoring started and for having hosted me in her lab for an intense, but special week. I appreciate her perseverance and her love for the sea.

I thank Prof. Roberto Sacchi both as coordinator of the Ph.D. program and as external examiner of this project: I really appreciated our discussions about statistical analyses.

Thank you, Prof. Federica Costantini and Dr Joachim Langeneck for your role as external examiners of the Ph.D. project. Your support and tips helped me a lot. Moreover, thanks to the External Reviewer of this Thesis, Prof. Federica Costantini and Prof. Giorgio Mancinelli.

A special thanks to Dr Romano Ambrogi for his for the critical review of the manuscript and his advices to improve the research paper on the second aim.

I would like to thank Federica and Giovanni for the pleasant time spent together, for the laughter and for the conversations on our future.

Thanks a lot to all the colleagues of the Botanical Garden. We spent a lot of funny time together, although the pandemic situation.

Finally, a special thanks to all the students that helped me both in logistic support during sampling surveys and laboratory activities: Alessandro Migliavacca, Alessandro Piazza, Alice Grioni, Chiara Mondaini, Cristina Cremonesi, Elisa Bigarella, Federica Gola, Gianmarco Riva, Giulia Quaini, Laura

Francia, and Lorenzo Pizzamiglio. A special thanks to Francesco Festa Marzotto, Laura Piazzese and Marcella Lo Vullo for her invaluable contribution to reach the second and third aims of this project.

We are grateful to: Municipality of Porto Venere, Port Authority of the Eastern Ligurian Sea, Scuola di Mare Santa Teresa, Cantiere Valdettaro, Assonautica La Spezia, Circolo Nautico Livorno, Yacht Club Livorno and Porticciolo del Chioma for granting permissions for these studies. Matteo Nannini and Dr Andrea Bordone (ENEA La Spezia) for the logistic support and the temperature dataset; Prof. Carlo Pretti, Dr Matteo Oliva and Dr Roberto Silvestri for the logistic support in Leghorn; Prof. Alfonso Ángel Ramos Esplà (University of Alicante) and Dr Joachim Langeneck (University of Pisa) for confirming the taxonomic identification of some ascidian and polychaete species, respectively, Ilaria Cabrini and the fundraising team “Universitiamo by UniPv”; Boldarino S.P.A and Curtiriso S.R.L and all the other donors and sponsors of the project; and Andrea Taccani (University of Pavia) for invaluable support in communication actions

Thank you again to Dr Jasmine Ferrario, both for her fundamental contribution for the achievement of these aims and for her ongoing support to me. We have been a good team!

Thanks to my beloved family: dad mum and sisters. Our complicity is still alive, despite I “left the nest. Your teachings are still fundamental.

Thanks to my “new” relatives, Monica and Renato, and Francesco and Gabriele, for their love and fun times spent together.

I had never been able to reach this achievement without my wife Laura, who has never stopped believing in me. As a new family, we are creating something unique and special. Thank you, my love.

*“Non si può essere infelici quando si ha questo: l’odore del mare, la sabbia sotto le dita, l’aria, il vento” (Irène Némirovsky)*