Expanding on the portuarization syndrome from an ecological perspective: eDNA reveals rich diversity, non-indigenous hotspots, and biotic homogenization in ports

Authors

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Abstract

Ports are well-known entry points for marine non-indigenous species (NIS), which arrive as hitchhikers on ships. Ports are also expected to be gateways for the spread of NIS in the wild and resemble each other more than communities outside due to their singular characteristics. However, the uniqueness of species assemblages in ports and how they differ from natural habitats have only been marginally investigated, notably at regional scale. Using eDNA metabarcoding, we obtained a comprehensive and standardized overview of metazoan community diversity in 12 paired ports and adjacent natural areas along the northwestern Mediterranean Sea. As expected, we found that NIS are more abundant in ports than in natural habitats, and that the species assemblages in ports differ from those in natural habitats. In addition, we observed that communities in ports are far more homogeneous than their natural counterparts. This finding supports the hypothesis of biotic homogenization in highly anthropized habitats. We also observed a pattern that had previously been documented mainly in fish, but that we identified here in every phylum studied except Arthropoda: species richness detected in ports is comparable to, and in some case even greater than, that observed in natural habitats. Overall, our findings broaden, through an ecological perspective, the “portuarization syndrome” concept, which originally defined ports as unique replicated environments that promote specific evolutionary processes.

Keywords

biological invasions, biotic homogenization, ports, artificial habitat, coastal habitats, metabarcoding

Introduction

Since the beginning of the nineteenth century, urban areas and megacities have increased in size and number as part of the urban transition (Day et al., 2021), notably affecting coastal zones (Creel, 2003; Day et al., 2021). Despite their defined borders, urban areas significantly impact the surrounding environment (Elmqvist et al., 2013) and create modified or entirely new habitats. The ecological and evolutionary processes of these habitats are only just beginning to be understood through the field of "urban science" (McKinney, 2002; Rivkin et al., 2019; Szulkin et al., 2020), which has been so far largely focused on terrestrial ecosystems. However, coastal urbanization substantially impacts marine coastal habitats and ecosystems (Aguilera et al., 2020; Bishop et al., 2017; Mayer-Pinto et al., 2018), which calls for strengthening marine urban sciences (Rega-Brodsky et al., 2022; Todd et al., 2019). In addition to their key ecological roles, coastal ecosystems support numerous economic sectors, including fisheries, tourism, wind energy production, and mineral resource extraction (Martínez et al., 2007; Seitz et al., 2014; Sempere-Valverde et al., 2022), leading to the worldwide proliferation of ports (Jouffray et al., 2020).

Ports provide new marine habitats characterized by particular abiotic properties (Alter et al., 2021; Touchard et al., 2023). For example, due to intense human activity and their (semi-)enclosed structure, they are characterized by a high concentration of waste and pollutants, stagnant water, turbidity and an irregular light regime (Cassi et al., 2008; Cutroneo et al., 2017; Heery et al., 2017; Luna et al., 2019; Schaefer et al., 2025). In contrast, their biotic properties remain largely unexplored (Madon et al., 2023), though ports are recognized as hotspots of biological introductions, with a high proportion of non-indigenous species (NIS) (e.g. Ferrario et al., 2017; O'Shaughnessy et al., 2020). This issue is noteworthy since the escape of NIS from ports is recognised as a primary vector of their spread into the natural environment (Afonso et al., 2020; Zenetos & Galanidi, 2020). Nevertheless, NIS occurrence in natural habitats surrounding ports has only been marginally investigated (Diem et al., 2023; Sghaier et al., 2019; Soares et al., 2018; Zarcero et al., 2024) as compared to their occurrence in ports (e.g. Tempesti et al., 2020). Yet, it could provide useful information for better understanding the conditions under which NIS expand their range from their primary sites of introduction.

Other biotic features of port ecosystems have been linked to reduced richness of “resident” species (i.e., term used hereafter to refer to species not classified as NIS) (Tanasovici et al., 2025): a pattern often explained by the low tolerance of many species to anthropogenic disturbances (Chapman, 2003; Chatzinikolaou et al., 2018; Schaefer et al., 2025). So far, fish seem to be the only exception. Fish biodiversity tends to be higher in ports than in nearby natural habitats because fish use ports to forage, escape predators, and establish nursery grounds (Bouchoucha et al., 2016; Manel et al., 2024). Despite this exception, port communities are generally considered to consist primarily of stress-tolerant, synanthropic, and non-indigenous species, which are “filtered” by port abiotic properties (Tempesti et al., 2020). This may result in communities within ports that resemble each other more than communities outside (i.e., biotic homogenisation, Olden et al. 2004, Todd et al., 2019). This global consequence has only been marginally explored (Lokatis & Jeschke, 2022; McKinney & Lockwood, 1999). The singularity of ports, acting as replicated environments, led to the definition of the “biological portuarization” syndrome (Touchard et al., 2023), defined as “the evolution of marine species in port ecosystems under human-altered selective pressures”.

The present work aims to further investigate the portuarization syndrome through an ecological perspective on animal marine communities and explore the aforementioned gaps, by comparing ports and their nearby natural habitats at a regional scale in the northwestern Mediterranean Sea. Due to the growing recreational boating industry, the number of small ports in the Mediterranean Sea has increased in recent years. Nowadays, there are more than 900 marinas in this basin, over half of which located in the northwest Mediterranean Sea (Carreño & Lloret, 2021). In our study area along the coast of France (continental and insular, including Corsica), 65 recreational ports with 39,900 berths were reported in 2018 (MTES, 2018), providing numerous sailing opportunities and port connections. Mediterranean ports are indeed characterized by intense maritime traffic connecting them at various scales (Ferrario et al., 2024; Ulman et al., 2019a). Additionally, previous studies have shown the high prevalence of NIS in these ports (Ulman et al., 2017). However, the diversity of resident species has been less investigated than that of NIS, leaving a gap in our understanding of the overall biodiversity inhabiting these ports. This partly stems from the traditional visual methods used for investigating marine biodiversity, which often involve a trade-off between depth and breadth. This restricts investigations to either a few selected taxa across multiple sites or to multiple taxa in a limited spatial context. Environmental DNA

83 (eDNA) metabarcoding is a standardized approach that can overcome some of these limitations and
84 complement or substitute traditional methods for biodiversity assessment and NIS detection (Couton et al.,
85 2019; Fonseca et al., 2023; Keck et al., 2022; Stat et al., 2017).
86 We used eDNA metabarcoding of seawater samples to assess the diversity and composition of animalia
87 communities in 12 pairs of northwestern Mediterranean ports and neighbouring natural areas. Based on the
88 previous studies cited above, we tested the following hypotheses: species assemblages in ports differ from
89 those in natural habitats (H1) and show biotic homogenization across a regional scale (H2); communities in
90 ports are characterized by greater abundance of NIS (H3), lower species richness than natural habitats (H4),
91 and taxonomic and functional traits distinct from communities from the nearby natural habitats (H5).

92 Materials and Methods

93 Data collection

94 Seawater samples used for this study were collected, during summer 2021 (June-August) within the framework
95 of the SUCHIMed campaign (https://doi.org/10.17600/18001619), in seven small ports (i.e., marinas) along the
96 French Mediterranean continental coast and five along the Corsican coast (Figure 1A; Supplementary material).
97 Twelve sites in natural habitats, outside each study port, were also sampled at the same time. Seawater was
98 collected in triplicates from three pontoons spanning the port and from a boat in three spots within a few
99 hundred meters from its mouth. Two litres of seawater were collected at each sampling site over a maximum
100 depth of 50 cm and immediately filtered on site or on boat by using 0.22 µm Sterivex filters and a VAMPIRE
101 peristaltic pump (Bürkle GmbH, Deutschland). Filters were filled with 2 mL of preservation buffer (sucrose 0.75
102 M, Tris 0.05 M pH 8, EDTA 0.04 M) and stored at -20°C until DNA extraction. From the boat and in the ports,
103 field controls were obtained by filtering ultrapure water with the equipment used on site. In total, 72 samples
104 were collected across the 12 localities.
105 DNA was extracted from Sterivex filters using the NucleoSnap® Finisher Midi extraction kit (Macherey-Nagel)
106 following the protocol detailed in Supplementary Protocol 1 in Couton et al. (2022). Fragments of COI, 18S rRNA
107 and 12S rRNA genes were each amplified 9 times from each DNA sample. Information about primers sequences,
108 PCR conditions and library preparation are reported in Supplementary material. PCR libraries were sequenced

(2 x 250bp paired-end) by Novogene company, using NovaSeq 6000 SP Reagent Kit (500 cycles) and a Novaseq 6000 SP flow cell sequencer. Negative controls were included both during extraction and library preparation.

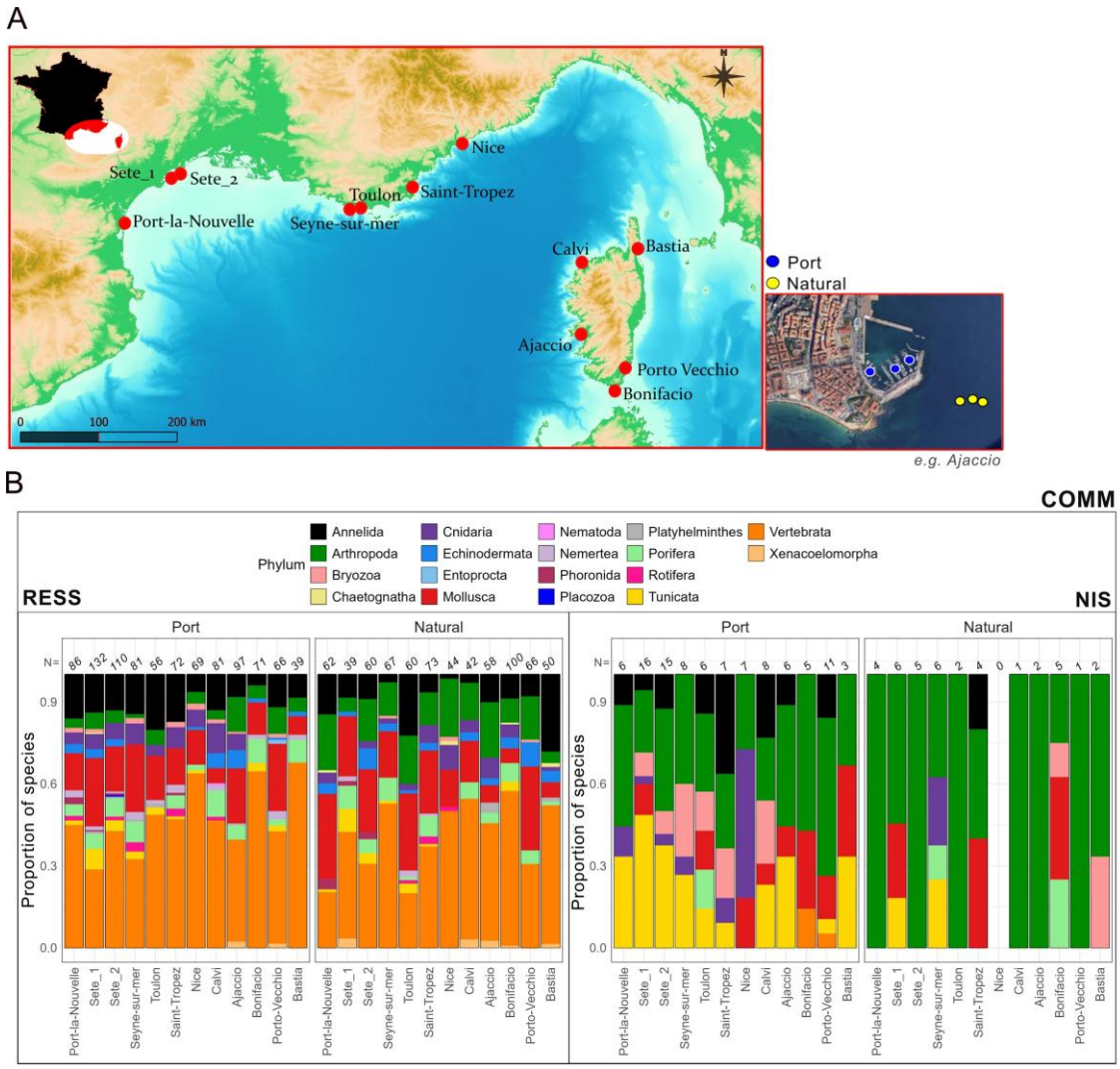

Figure 1: A) French localities where seawater was collected at three sites inside ports and three sites in nearby natural habitats. An example is shown for Ajaccio in the small panel. Basemap: GEBCO_Grid, colour-shaded for elevation (GEBCO Compilation Group, 2023); B) Proportion of resident species (i.e., autochthonous; RESS) (left panel) and NIS (right panel) per phylum per locality and habitat type (i.e. Port vs Natural habitat). The number of RESS and NIS recorded in each locality and habitat is reported at the top of each bar.

Bioinformatic analyses and taxonomic assignment

Paired-end raw reads were pre-processed using cutadapt v1.16 and bbMap v38.22, followed by a modified Unix shell script based on Brandt et al. (2021) (https://gitlab.ifremer.fr/abyss-project/abyss-pipeline) for denoising

120 (DADA2 v1.10;Callahan et al., 2016) and clustering (Swarm v3.1.0 with d=1 for 12S and 18S and d=3 for COI;
121 Mahé et al., 2014). OTUs tables were decontaminated using the package decontam (Davis et al., 2018) and a
122 correction for tag switching was applied (Wangensteen et al., 2018). For COI data, mumu (Mahé, 2023, adapted
123 and modified from Lulu, Frøslev et al., 2017) was applied to minimize the bias due to the existence of paralogs
124 and pseudogenes (particularly nuclear degenerated copies, *numts*). OTUs sequences were aligned with those
125 in reference databases using BLAST v2.12.0 (Altschul et al., 1990). Sequence coverage was set at a minimum of
126 70% for 12S and 80% for COI and 18S. Minimum identity percentages with sequences in reference databases
127 were set according to Couton et al., (2022): 92% for COI and 90% for 12S (MIDORI2 database) and 99% for 18S
128 (Silva v138 database).
129 OTU tables were rarefied to the minimum number of reads per sample (i.e., 44,798 for COI, 107,876 for 18S,
130 44,798 for 12S). WoRMS package version 0.4.3 in R (Chamberlain & Vanhoorne, 2023) was used to correct the
131 nomenclature and remove non-marine taxa; chordates were split into Vertebrata and Tunicata due to the
132 important ecological differences between these two subphyla.
133 The OTU tables from the three markers were combined to generate a non-redundant taxonomy table. Taxa not
134 resolved to the species level with a particular marker were removed if they co-occur in the same sample with
135 an OTU assigned with higher resolution with another marker; otherwise, they were retained. The final dataset
136 (i.e., Species occurrence table) was converted to a species-level presence/absence matrix, excluding all OTUs
137 not identified at species level, to enable determining the NIS vs. resident status.
138

139 NIS identification

140 The final dataset including all OTUs identified at species level (hereafter "COMM" dataset, i.e. whole
141 community) was screened to identify non-indigenous species (NIS), by using the NIS database for France
142 established by Massè et al (2023). The ANIS-E database (Violet et al., *submitted*), which is a custom database
143 including all NIS reported in European Seas from 1492 to 2023, was also used for identifying NIS reported in
144 Europe. Species found in these databases were classified as "Non-Indigenous-Med Sea" when reported as NIS
145 in the Mediterranean Sea and "Non-Indigenous-Europe" when reported as NIS in other European Seas and not
146 native to the Mediterranean Sea. Species not found in these databases were classified by default as resident

species (RESS). RESS included species native to Mediterranean Sea, albeit some species presumably from elsewhere but not yet reported as NIS in Europe may be included as well. We did not further screen the RESS for potential novel NIS because of the risk of false positives (false NIS identification) when using metabarcoding alone (Couton et al. 2022).

Functional traits classification

Functional traits can help elucidate the ecological processes that determine the presence of certain species in specific environments (Mayer-Pinto et al., 2018) or those associated with biological invasions (Leclerc et al., 2023). Hence, we characterized species using two functional traits, “motility” and “ecology”, selected because they provide information about the dispersal potential of NIS (mobile NIS have greater dispersal potential than sessile ones) and the filtering effect of ports (ports can act as positive filters on species colonizing surfaces and inhabiting the benthos). Motility of species was coded as “sessile” and “mobile”. Ecology was coded for fish using Fishbase (Froese & Pauly, 2000), following Rey et al., (2023) with slight modification to get a more even distribution, as follows: “demersal” and “bathydemersal” species were grouped into “demersal”, and “pelagic-neritic”, “pelagic-oceanic” and “bathypelagic” species into “pelagic”; “benthopelagic” and “reef-associated” classes were left unmodified. For other species not classified as fish, the modalities for the “ecology” trait were retrieved from the Functional group section available in WoRMS (WoRMS Editorial Board, 2025): species classified as “benthos” and “benthos> macrobenthos” at adult stage were reported as “benthos”; species classified as “plankton”, “plankton>zooplankton” and “nekton” (only *Tursiops truncatus*) at adult stage were classified as “pelagic-non-fish”. An additional trait was considered for fishes, namely “Fishing vulnerability”, which could be related to the greater occurrence of fishes in ports, because of fishing restriction in ports and presence of fishing discards (from recreational fishing boats moored in the port). Vulnerability scores were retrieved from FishBase, where this trait is expressed as a numerical value from 0 (low vulnerability) to 100 (high vulnerability).

Diversity analyses

173 Diversity analyses were implemented in R studio (R Core Team, 2021). With presence-absence data, alpha-
174 diversity was estimated by species richness. As we expected to have more NIS (H3) but lower species richness
175 in ports (H4), t-tests or Mann Withey tests were performed. NIS proportion and richness were also examined
176 with one-way ANOVA (or Kruskal-Wallis test) between localities.
177 For beta-diversity analyses (H1 and H2), the Jaccard dissimilarity was used. Principal Coordinates Analyses
178 (PCoA) was used to visualize community's dissimilarities in an unconstrained ordination space. The possibility
179 that communities from ports and natural habitats displayed distinct species composition (H1) was tested
180 performing Partial Distance based Redundancy Analysis (dbRDA) on Jaccard dissimilarities with habitat type as
181 explanatory variable; to control for potential effect of locality, this factor was included in the partial dbRDA as
182 covariate. The statistical significance of this constrained ordination was assessed by ANOVA-like test
183 (*anova.cca*() function of vegan package). In addition, nestedness and turnover components of beta diversity
184 were estimated using the R package betapart v. 1.6 (Baselga et al., 2023) to define whether differences in
185 species composition between habitats were driven by species gain/loss or species replacement. Permutational
186 Analysis of Multivariate Dispersion (*PERMDISP*) was performed through the *betadispers*() function from the
187 vegan package in R (Oksanen et al., 2013) to test for different dispersion of Jaccard dissimilarities between ports
188 and between natural habitats. The hypothesis of greater biotic homogenization in ports (H3) was considered
189 verified if, in case of significant PERMDISP, this habitat type showed smaller dispersion of Jaccard dissimilarities
190 between samples than natural habitats.
191 To examine the influence of taxonomical and functional traits in determining species composition in different
192 habitats (H5), species significantly associated with either type of habitat were identified by Indicator Species
193 Analysis using R package indicspecies (De Cáceres & Legendre, 2009). In addition, species scores from the partial
194 dbRDA first axis were compared between taxonomical (i.e. Phylum and Class) and functional (i.e. motility and
195 ecology) traits by either t-tests or ANOVAs (or their non-parametric equivalents) depending on number of levels
196 of the trait. In case of significant ANOVA, post-hoc Tuckey Test (or their non-parametric equivalents) with
197 Benjamini-Hochberg ("*bh*") correction were implemented for pairwise comparisons between trait levels.
198 Spearman rank correlation was performed between the distribution of fish species scores along the first partial
199 dbRDA axis and values of fishing vulnerability.

200 # Results

201 The outcome of the bioinformatics pipeline analysis is provided in the Supporting Information. The combination
202 of the three markers (Supplementary Figure 1) allowed to identify 538 taxa at the species level (i.e., Species
203 occurrence table) belonging to 18 phyla (Figure 1B). An average of 125 ± 23 (mean ± SE) species was recorded
204 in each locality (Figure 1B).

205 Out of the 538 species (i.e. whole community, COMM), 497 were classified as resident species (RESS), of which
206 327 were known as native to the Mediterranean Sea, leaving 120 species with unknown origin, possibly
207 including unreported Non-Indigenous species (NIS) in European seas. Finally, 41 species were identified as
208 unequivocal NIS, meaning they had previously been reported as such in Europe. Of these, eight were
209 cryptogenic and five had been reported as NIS in European Seas though not yet in the Mediterranean Sea
210 (Figure 2). The average percentage of NIS per locality (regardless of habitat type) was 6.8± 4.6% (mean± SE),
211 reaching up to 12.6 ± 2.03% and 9.7 ± 3.7% in Sete_1 and Sete_2, respectively (Supplementary Figure 2). This
212 contribution was not significantly different across the 12 localities (Kruskal-Wallis test, P value = 0.07).

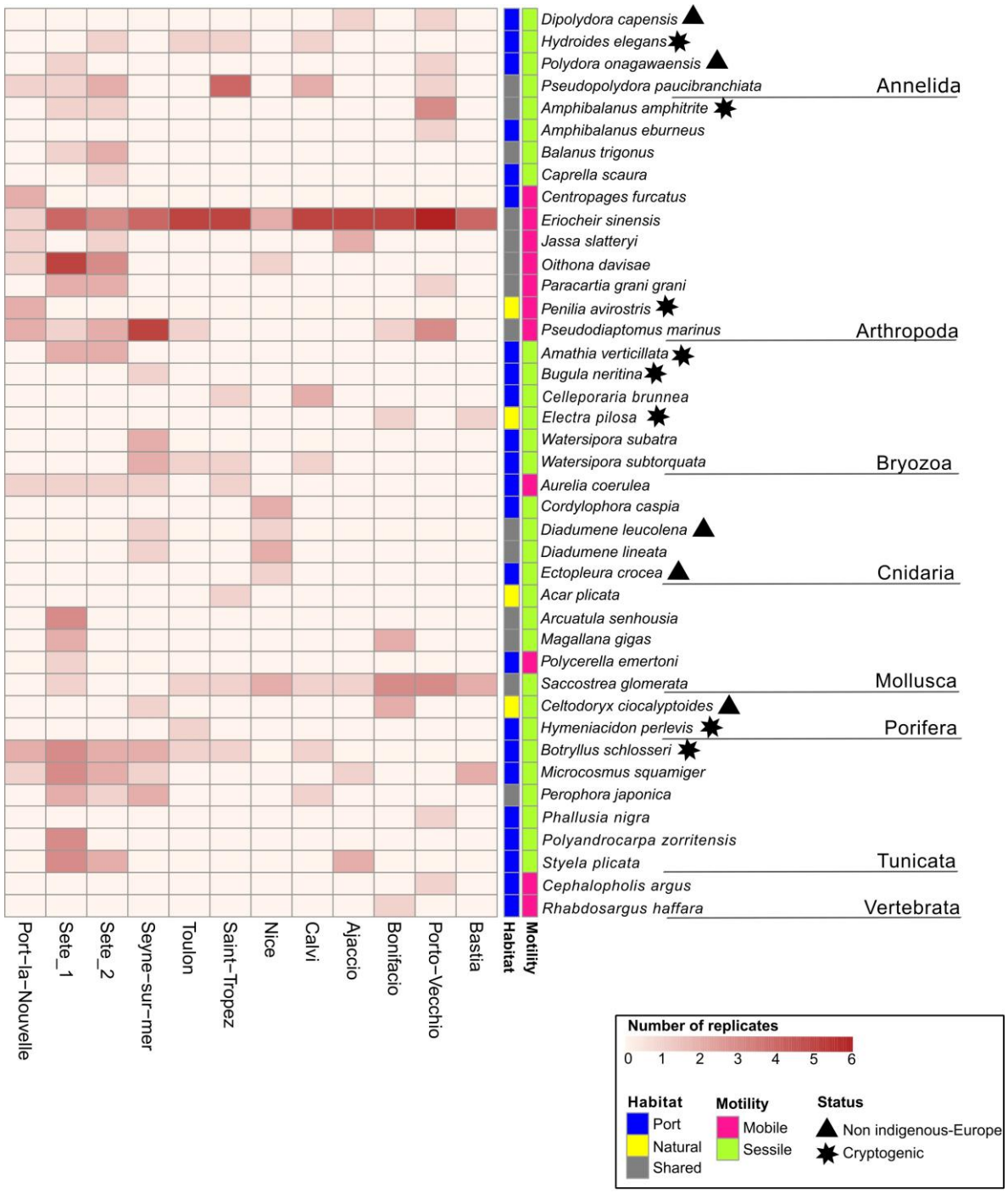

Figure 2: NIS (n=41) presence per locality and attributes. The heatmap displays the number of replicates (out of n=6, with 3 inside and 3 outside ports) where each NIS was recorded. Coloured bars on the right display habitat and motility trait for each NIS. Symbols are added to highlight their status as cryptogenic (star) and/or as NIS observed in Europe but not in yet in the Mediterranean (triangle).

218 *Ports display species assemblages different from those in natural habitats and homogeneous at a regional*
219 *scale*

220 Across the sampling range, communities from ports were distinct from those in natural habitats (as reported
221 by unconstrained and constrained ordinations respectively in Supplementary Figure 3A and Figure 3A). This
222 observation was supported statistically by ANOVA.cca on partial dbRDA on habitat type (F-statistic=2.70, p-
223 value = 0.001; R^2-adjusted=2.73%), thus confirming H1. The observed dissimilarities were mainly driven by
224 species replacement in the two habitats (turnover) rather than by species loss in either one of the habitats
225 (nestedness) (Figure 3B). Furthermore, lower dispersion of Jaccard dissimilarity values were observed for ports
226 than for natural habitats (Figure 3C), supporting the hypothesis of greater biotic homogenization in ports than
227 in natural habitats (H2) (*PERMDISP*, p-value = 0.001).

228 Testing H1 and H2 on RESS and NIS separately revealed a different behaviour of the two communities. Both
229 communities displayed different species in ports than natural habitats (Supplementary Figure 3B/C), and H1
230 was statistically confirmed by ANOVA.cca on partial dbRDA (RESS: ANOVA.cca on partial dbRDA: F-
231 statistic=2.63, p-value = 0.001; R^2-adjusted=2.6%; NIS: ANOVA.cca on partial dbRDA: F-statistic=4.17, p-value
232 0.001; R^2-adjusted=5.35%; Supplementary Figure 4A). However, RESS dissimilarities were driven by turnover
233 while those of NIS have an even contribution of both beta-diversity components (Supplementary Figure 4B).
234 Biotic homogenization in ports (H2) was confirmed for RESS (*PERMDISP*, p-value = 0.004) but not for NIS
235 (*PERMDISP*, p-value = 0.18) (Supplementary Figure 4C).

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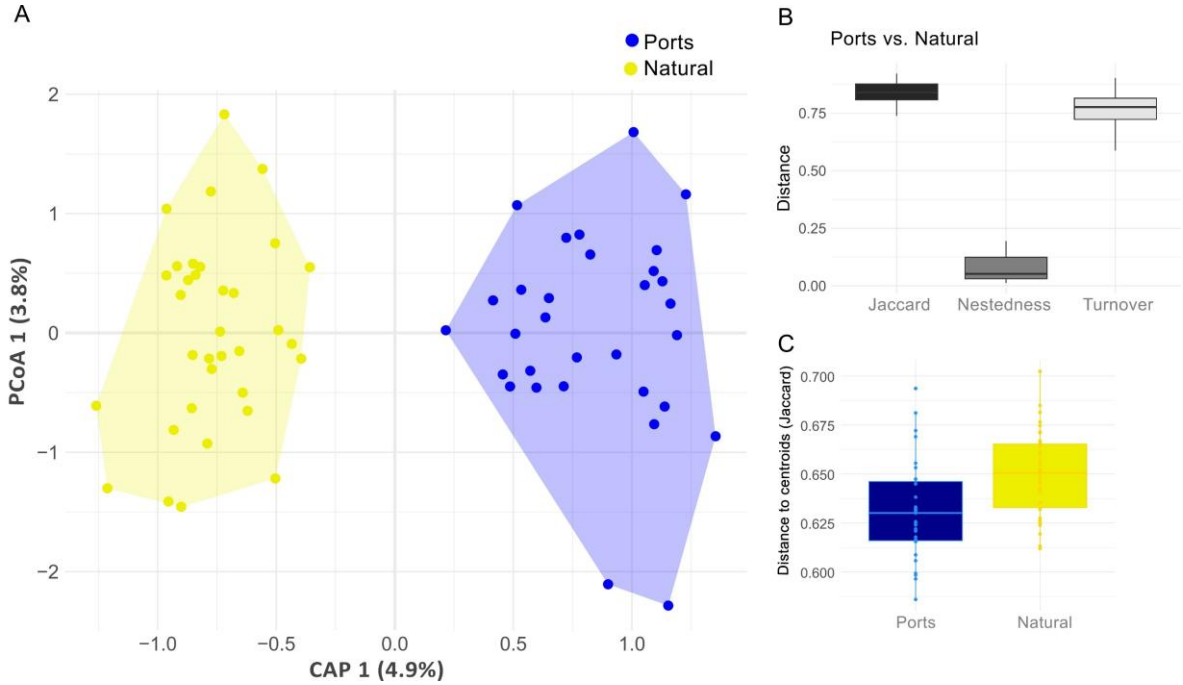

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238 *Figure 3: Jaccard dissimilarities based on species composition of communities (COMM) in ports and in natural*
239 *habitats. A) Partial dbRDA performed to test the effect of habitat on COMM species composition accounting for*
240 *the effect of locality. The analysis produced one constrained axis (CAP 1) and an unconstrained one (PCoA 1) due*
241 *to the inclusion of only one explanatory variable; B) boxplots displaying partitioning of Jaccard dissimilarities in*
242 *its nestedness and turnover components with comparisons between samples from inside ports and from natural*
243 *sites; C) boxplot showing Jaccard dissimilarities between samples collected inside ports (blue) and in natural*
244 *habitats (yellow).*

245

246 ***NIS are overrepresented in ports compared to natural habitats***

247 In line with our working hypothesis (H3), the proportion of NIS in ports (9.2 ± 3.7%) was more than twice that
248 in natural habitats (4.6 ± 3.5%). With two exceptions (Bonifacio and Sete_1, Figure 4), this trend was observed
249 in every locality and was significant in three of them, namely Seyne-sur-Mer, Porto-Vecchio and Nice (in the
250 later there was no NIS outside the port).

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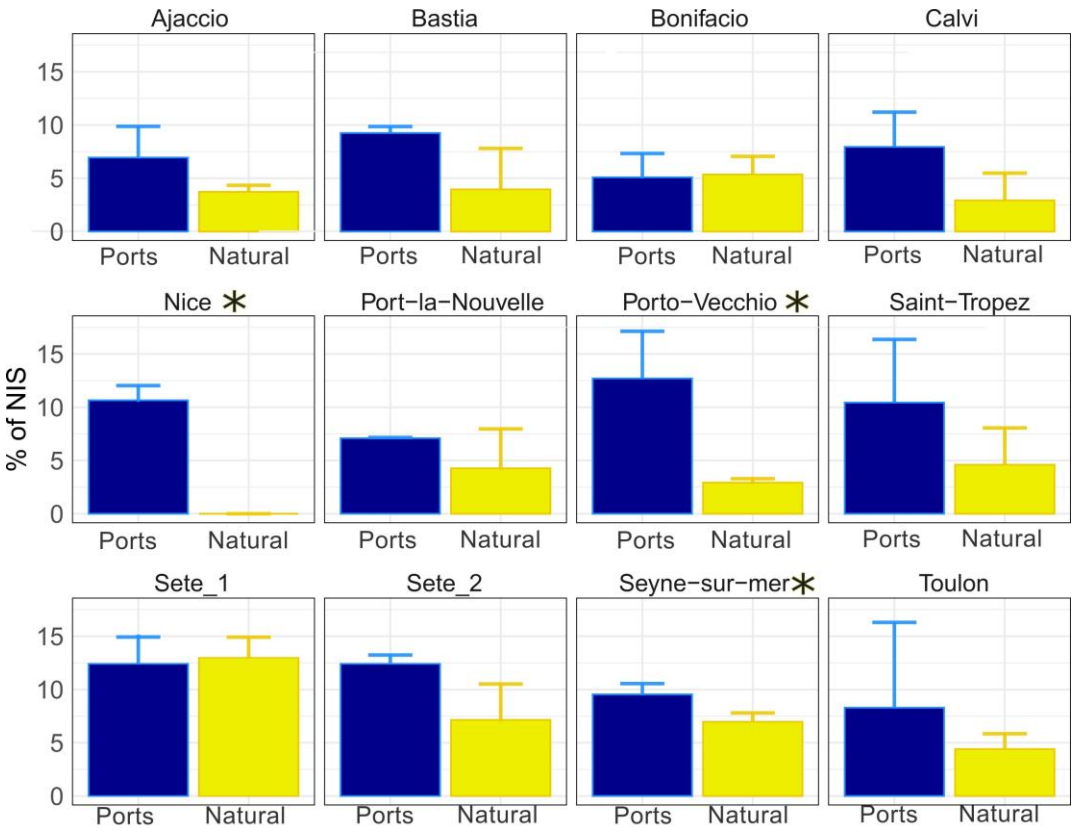

*Figure 4: Barplot showing mean and standard error of the percentage of NIS in communities sampled in ports and in natural habitats in each locality. Localities that showed a significant difference in the proportion of NIS inside and outside marinas are labelled with * (Mann-Whitney test, one-tailed, P value = 0.01 for Porto-Vecchio and Seyne-sur-mer; no NIS recorded outside Nice marina).*

The number of NIS was also higher in ports across all localities (Mann Whitney tests, one-tailed, p-value < 0.01) (Figure 5) and per locality (Supplementary Figure 5). In addition, six times more NIS were observed exclusively in ports (23 NIS) than in natural habitats (4 NIS) (Figure 5). It is however worth noting that ca. 40% of NIS detected in ports were also present in natural habitats (14 NIS; Figure 5).

The greatest NIS richness inside and outside ports was found in both Sete ports (Sete_1: average 10 ± 2 NIS inside and 4 ± 1 NIS outside; Sete_2: average 11 ± 2 NIS inside and 3 ± 1 NIS outside).

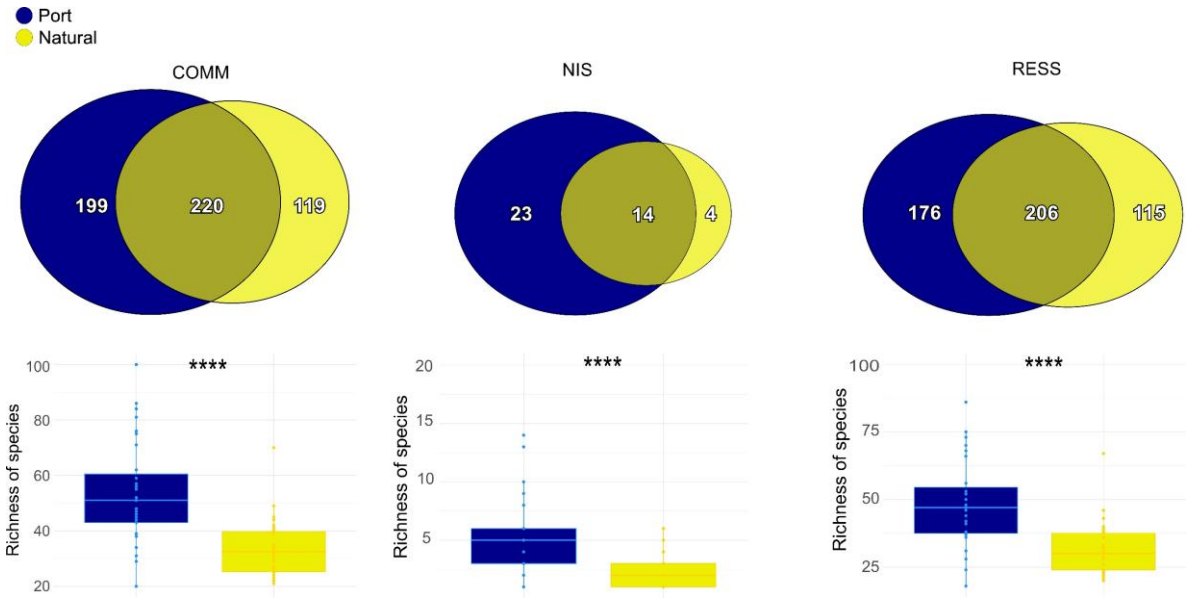

Figure 5: Venn diagrams showing the total number of species exclusive or shared in ports and natural habitats, considering the whole community (COMM), non –indigenous species (NIS) only and resident species (RESS) only. Boxplots of species richness per sample calculated for each habitat type are also provided; significant difference between the two habitats is indicated with asterisks (Mann-Whitney test, P value < 0.001).

Ports host rich communities regardless of high NIS occurrence

Contrary to our working hypothesis (H4), ports considered in this study did not display lower species richness than nearby natural habitats (COMM, Figure 5, Supplementary Table 1). In fact, the reverse was true overall (Mann-Whitney test, p-value < 0.001). Higher richness inside port was also observed in each locality separately (Supplementary Figure 6), with significant differences in Calvi, Seyne-sur-Mer and Porto-Vecchio (Supplementary Table 1). Considering phyla separately, H4 was confirmed only for Arthropoda (Mann-Whitney, p-value = 0.04; Supplementary Figure 6); instead, nine phyla displayed greater species richness in ports than in natural habitats: Vertebrata, Tunicata, Bryozoa, Cnidaria, Annelida, Porifera, Nemertea and Rotifera (Supplementary Table 1 and Supplementary Figure 6).

The above-mentioned observations were not driven by the higher NIS occurrence in ports: testing H4 on RESS community alone produced similar results of higher richness in ports that nearby natural habitats (Figure 5) also across all locations (p-value > 0.05 for all comparisons).

284

285 *Taxonomical and functional traits drive differential species occurrence in ports and natural habitats*

286 Community dissimilarities between ports and natural habitats were explained by the differential occurrence of
287 60 species (including RESS and NIS) identified by Indicator Species Analysis (IndVal.g, p-value < 0.05,
288 Supplementary Table 2, Figure 6A). A higher number of species (53) are indicators of ports than of natural
289 habitats (7 species), suggesting again a more homogeneous and distinct community in the former. Most
290 indicator species were Teleostei (33.9% of indicator species of ports and 42.8% of natural habitats), followed
291 by Polychaeta in ports (18.8%) and Copepoda (42.8%) in natural habitats. NIS represented 16% of indicators of
292 ports while none was significantly associated with natural habitats (Figure 6A).

293 The distribution of species scores along the first axis of the partial dbRDA was analysed to determine if
294 differences in species occurrence between habitats were related to taxonomic or functional traits (Figure 6B).
295 Species scores were differentially distributed along the axis according to Phylum (Kruskal-Wallis test, p-value =
296 0.002), Class (Kruskal-Wallis, p-value =0.04), motility (Mann-Whitney test, P value = 0.003), ecology (for non-
297 fish species, Mann-Whitney test, p-value < 0.001; for fish species, Kruskal-Wallis test, P value = 0.03) and their
298 NIS status (Mann-Whitney test, P value = 0.002) (Supplementary Table 3). In contrast, no significant correlation
299 was found between fishing vulnerability and fish species scores along the first dbRDA axis (Spearman
300 correlation, p-value = 0.06), suggesting no relationship between the two factors.

301 As reported in Figure 6B and Supplementary Table 3, Vertebrata and Tunicata tended to be associated with
302 ports (i.e. species scores displaying positive values of dbRDA axis), and Arthropoda and Echinodermata with
303 natural habitats (i.e. negative values of dbRDA axis) (Post-hoc Dunn tests, p-value < 0.05). At Class level, species
304 in ports were mostly classified as Teleostei and Ascidiacea while those outside as Copepoda. Ports were also
305 associated with sessile and benthic non-fish species, as well as with demersal and benthopelagic fishes. In
306 contrast, mobile species, pelagic-non-fish and fishes classified as “reef-associated” and “pelagic” characterized
307 natural habitats.

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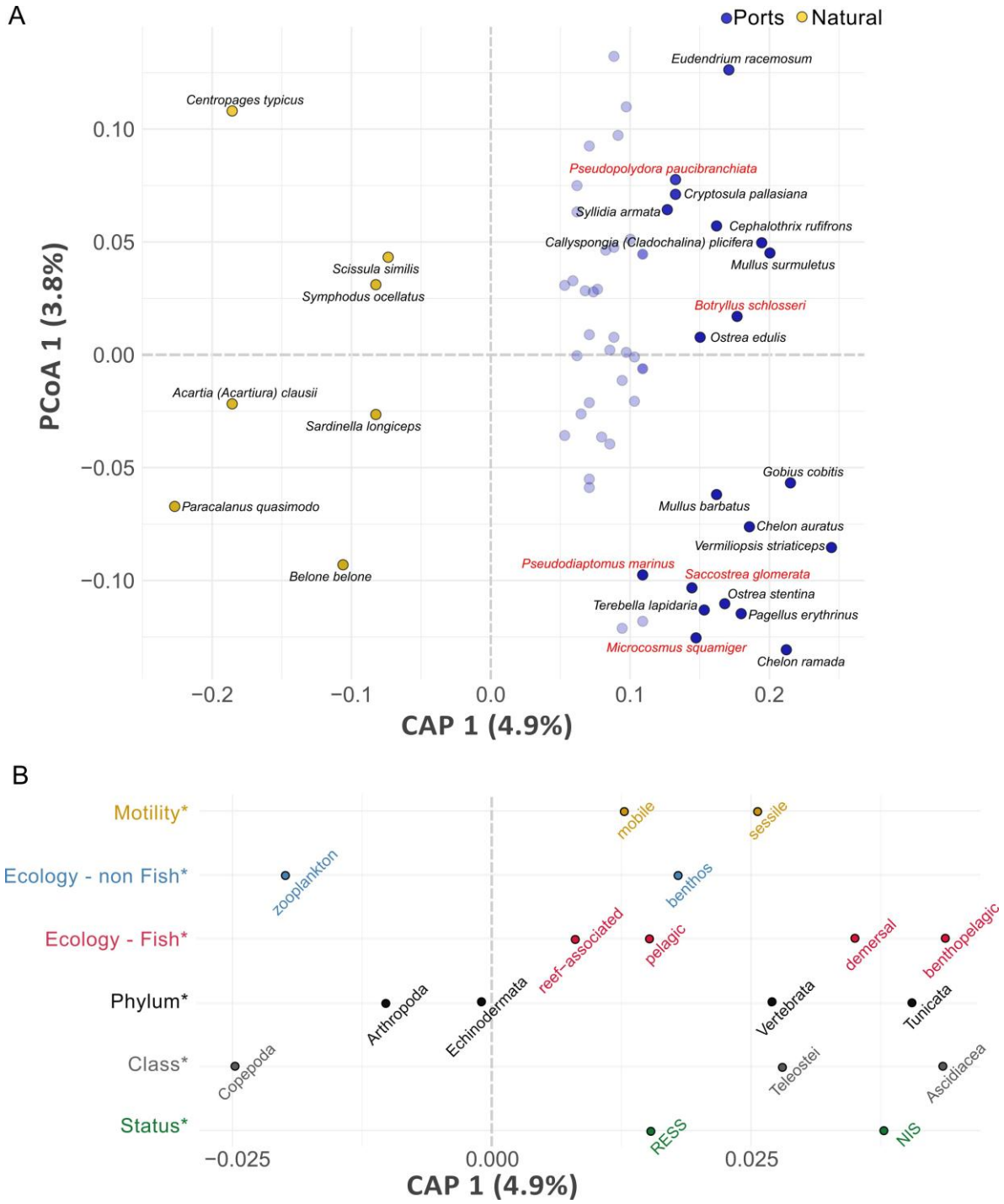

Figure 6: Species scores of the partial dbRDA performed to test the effect of habitat on COMM species composition accounting for locality; A) species scores of the 60 indicator species identified for each habitat by Indicator Species Analysis: species labels are displayed for all 7 indicator species of natural habitats (yellow) and only for the 20 indicator species of ports (blue) displaying the highest statistics in the permutation test (Inval.g);

NIS are reported in red; B) average values of species scores of partial dbRDA first axis obtained for each functional and taxonomic group and for their classification as NIS and RESS: traits that significantly influenced the distribution of species scores in the first dbRDA axis are flagged with an asterisk (for tests performed, statistics and p-values see Supplementary Table 3). Values positioned toward the right side of the first dbRDA axis are more strongly associated with ports while those toward the left side with natural habitats.

As reported in Supplementary Table 3, testing the influence of traits on RESS dissimilarities yielded results similar to COMM, except for the phyla linked to each habitat type: only Vertebrata (Teleostei) and Arthropoda (Copepoda) were mainly found, respectively, inside and outside ports (Post-hoc Dunn test, > 0.05). In contrast, for NIS, no taxonomical or functional trait characterized NIS associated with either ports or natural habitats.

Discussion

Are ports acting as anthropogenic biodiversity reservoirs?

Using eDNA metabarcoding on 72 seawater samples collected in 12 pairs of ports and adjacent natural habitats along the French Mediterranean coast, we identified 538 animal species, including 41 NIS. Our study revealed marked differences between ports and natural habitats in community richness, structure and functional traits. Beyond the expected higher prevalence of NIS in ports, a striking and not anticipated result was the overall greater species richness in ports than in natural habitats across nearly all phyla.

Several factors may be considered to explain this observation. First, ports display a heterogeneous mosaic of substrates, with infrastructure most often built on formerly soft-sediment habitats. The variety of materials and structure used in ports creates a mosaic of microhabitats, likely suitable to support a diversity of species assemblages (Connell & Glasby, 1999; Romoth et al., 2023; Todd et al., 2019). Second, the nutrient enrichment in ports (García-Pintado et al., 2007; Guerra-García et al., 2021; Karakassis et al., 2005; Sebastiá & Rodilla, 2013) may enhance primary production (Scott, 1978), and, under certain conditions, secondary production (Leslie et al., 2005), thereby increasing taxonomic diversity (Whittaker et al., 2003), as observed for Polychaeta by Dafforn et al., (2013). Third, the (semi-)enclosed structure of ports may benefit some taxa by providing still

340 waters suitable for foraging and nursery grounds, particularly for fishes, for which ports are also no-take areas.
341 In some cases, fish communities are as diverse –or even richer- than those in natural habitats (Bouchoucha et
342 al., 2016; Lapinski et al., 2014; Manel et al., 2024; Pérez-Ruzafa et al., 2006). In addition, greater fish presence
343 may also exert top-down control on space-dominant competitors among epibiota, enhancing community
344 diversity (Rivero et al., 2013). Intermediate levels of disturbance, common in small ports such as marinas, may
345 further promote diversity by enabling the coexistence of both subordinate and dominant species (Boulanger et
346 al., 2021; Dial & Roughgarden, 1998; Santos et al., 2021; Turon et al., 2022).
347 Lastly, methodological aspects of our study could also partly explain our results: although seawater samples
348 were collected in natural sites with a maximum depth of 50 meters, marinas are shallower and retain water
349 longer, potentially amplifying the eDNA signal from benthic species compared to more open natural habitats.
350 This explanation is in line with the exception observed in arthropods (i.e., lower species richness in ports), which
351 are dominated by pelagic/mobile species. However, fish diversity was also greater in ports, and our findings
352 thus aligned with others comparing Operational Taxonomic Units between ports and sites located in no- take
353 marine reserves (Macé et al., 2024; Manel et al., 2024). This methodological aspect is thus unlikely to explain
354 the results obtained overall. Nevertheless, further targeted studies on substrate-specific assemblages should
355 be carried out to make a more detailed comparison between the two types of habitats.
356

357 *Port similarities and singularities: the ecological side of the "portuarization syndrome"*

358 We found a greater community similarity between ports than between natural habitats at a regional scale, a
359 finding supporting the hypothesis of biotic homogenization. This also allows broadening, from an ecological
360 perspective, the concept of "portuarization syndrome" proposed by Touchard et al. (2023), which defines
361 specific port-induced evolutionary processes resulting from their distinctive biotic and abiotic properties and
362 their replication across a large spatial scale. This homogenization likely results from two processes:
363 environmental filtering of autochthonous species (Todd et al., 2019) and high connectivity among them due to
364 boating, which uniformizes species assemblages across scales, notably NIS (Touchard et al., 2023; Ulman, et al.,
365 2019a; Ulman et al., 2019b). To our knowledge, this is the first study to provide evidences for biotic
366 homogenization in ports at a regional scale. The portuarization syndrome is also visible through species

assemblages in ports differing significantly from those in nearby natural areas. Interestingly, and in line with the reservoir effects discussed above, this dissimilarity between port and dissimilar habitats was not primarily due to simple species gains or losses (nestedness) between natural sites and marinas, but rather to extensive species turnover.

This pattern scales up to the Class level, with Teleostei and Copepoda exhibiting contrasted associations with the two habitats, as revealed by the species scores of partial dbRDA. By being predated both by fish larvae and adults, zooplankton populations are strongly regulated by fish (Gliwicz & Pijanowska, 1989). High fish density in ports may reduce copepods species richness, through species-specific and size-dependent selective predation (Fiksen et al., 2005; Lehtiniemi et al., 2007). Moreover, zooplankton is highly sensitive to water temperature, transparency and oxygen concentration (Stoch et al., 2023; Witalis et al., 2024), which are often altered in ports. This may further reduce the occurrence of zooplankton populations.

A second important factor distinguishing port communities from naturals sites was the studied functional traits, namely species motility and ecology. Functional trait analyses confirmed the expectation of a rich and abundant fouling community in ports (Tempesti et al., 2022) characterized by sessile and benthic species. The higher association of demersal and benthopelagic fishes than pelagic ones with ports was also expected given the reliance of the former species on benthic preys (Bergstad, 2019). In contrast, reef-associated fishes were less represented, likely reflecting the lack of complex surfaces (e.g. crevices) within ports.

Interestingly, this role of ecological filter of ports that appears clearly for RESS is not obvious for NIS. No specific beta-diversity component seems to drive NIS-community dissimilarities, with a balanced contribution of turnover and nestedness. While only a portion of NIS may have spread and establish in nearby natural habitats (15 shared detection across 42 NIS species), a little less than 10% (4) were detected only outside marinas. This may reflect the difficulties in detecting all species with a limited sampling volume in a rich substrate, or to the existence of alternative introduction vectors beyond shipping and boating – such as aquaculture, a particularly relevant vector in France (Massé et al., 2023). Besides, none of the study traits provided insights into the habitats where NIS were more likely to occur. Although it is challenging because many NIS are poorly studied (but see e.g., Leclerc et al. 2023), future trait-based analyses of NIS should expand beyond mobility and ecology

393 traits, to examine in particular tolerance to environmental conditions including pollutants, foraging strategies,
394 dispersal traits and growth rates, with the aim of better predicting their establishment and spread likelihood.
395
396 *Are NIS over-looked in the wild?*
397 Across all localities, 41 non-indigenous species (NIS) were identified including five not yet recorded in the
398 Mediterranean but known as NIS in other European Seas. Although it is not possible to confirm their presence
399 with eDNA only (i.e., false positives may occur, see Couton et al., 2022), our result identifies localities in which
400 further investigation should be prioritized for targeted molecular investigation and/or surveys based on direct
401 observation.
402 As expected, most NIS were found in ports (and 23 exclusively) while 18 occurred in natural habitats (4 being
403 private to them) and the same was observed for NIS proportions. Nevertheless, in contrast with the general
404 pattern observed, a high proportion of NIS was observed in the Thau lagoon (higher than found in some ports),
405 which is the natural site paired with the port at Sete_1 and also close to the port of Sete_2. Despite being
406 recognized for its rich environment – supporting its classification as "Site of Community Importance" under the
407 Habitat directive of the Nature 2000 framework –, the Thau lagoon is also a well-known introduction hotspot
408 in France. This is due to a massive import of Japanese oysters in the seventies (Boudouresque et al., 2010),
409 usually travelling with their associated species settled on shells and in the material used to collect and transport
410 spats. In addition, the Thau lagoon is surrounded by many small cities and their associated marinas, as well as
411 diverse recreational activities adding to the port's effect. This exemplifies the tights link between the diversity
412 of introduction vectors/pathways and NIS establishment (Ojaveer et al., 2018).
413 The proportion of NIS in ports, though generally higher, was however not dramatically greater than in natural
414 habitats, with only three localities (Seyne-sur-Mer, Porto-Vecchio, and Nice) showing a significant increase in
415 NIS proportions in port. So far, little attention has been given to natural habitats near ports, with NIS being
416 primarily investigated only inside ports (Madon et al., 2023; Ulman et al., 2017) or in distant and pristine sites
417 such as marine protected areas (Borriglione et al., 2025; Mannino et al., 2017). Our results suggest that natural
418 habitats adjacent to ports may play a critical but underappreciated role as stepping-stone locations for the
419 colonization of more distant and pristine habitats, especially if this transient establishment allows NIS to

acclimate or adapt to local natural conditions. Natural habitats nearby ports are likely to be under the influence of human activities. These disturbed conditions may lower competitive resistance from native species (Ardura et al., 2016; Giakoumi & Pey, 2017), making these habitats particularly vulnerable to secondary establishment. Therefore, it is crucial to survey NIS presence in these locations not only because it can inform about NIS capable to spread further but also because they represent the majority of coastal zones in the highly urbanized Mediterranean Sea.

Overall, our study revealed that ports are introduction hotspots harbouring unexpectedly high diversity across animal phyla forming rather homogeneous communities at the regional scale. This provides empirical support for the "portuarization syndrome". The substantial NIS presence in adjacent natural habitats simultaneously underscores the need for expanding monitoring and NIS studies beyond ports to better understand the spatial dynamics of marine bioinvasions and the interplay between the expanding urban sprawl and their natural counterparts in coastal zones.

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Data archiving statement

The codes and datasets used to perform the analyses will be provided upon acceptance of the paper.