Pollution gradient leads to local adaptation and small-scale spatial variability of communities and functions in an urban marine environment

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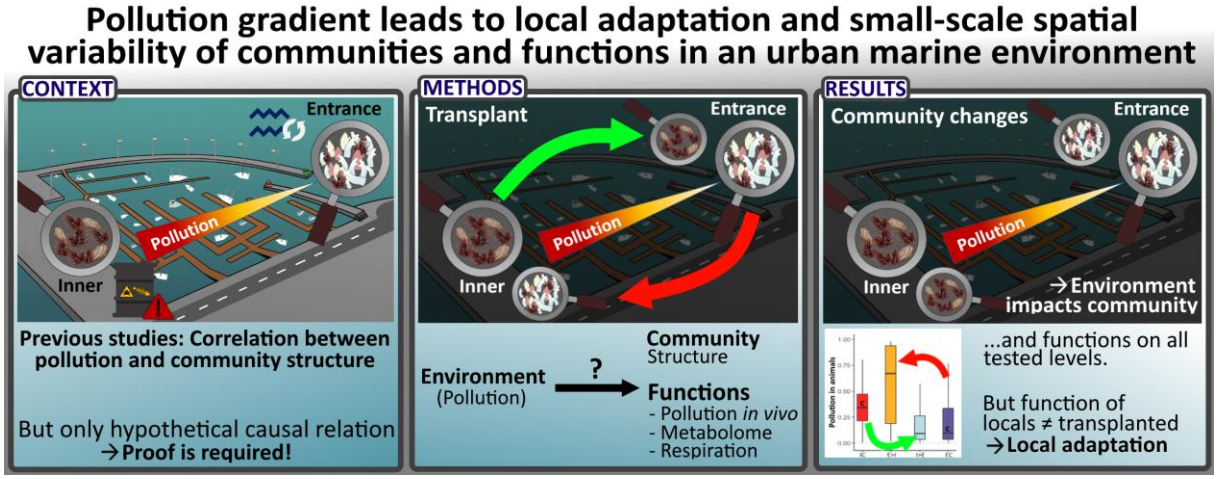
Abstract. Urbanization of coastal habitats, of which harbors and marinas are the paragon, has led to various ecological paradigms about their functioning. Harbor infrastructures offer new hard substrata that are colonized by a wide variety of organisms (biofouling) including many introduced species. These structures also modify hydrodynamism and contaminant dispersal, leading to strong disturbance gradients within them. Differences in sessile community structure have previously been correlated to these gradients at small spatial scale (< 100 m). Local adaptation might be involved to explain such results, but as correlation is not causation, the present study aims to understand the causal link between the environmental gradients and community structure through a reciprocal transplant experiment among three sites of a marina (inner, middle, entrance). Our results highlighted strong small-scale spatial variations of contaminants (trace metals, PCB, pesticides, and PAH) in sediments and animal samples which have been causally linked to changes in community composition after transplant. But historical contingency and colonization succession also play an important role. Our results provided strong evidence for local adaptation since community structure, respiration, and pollutant uptake in *Bugula neritina,* as well as the metabolomes of *B. neritina* and *Ciona intestinalis* were impacted by the transplant with a disadvantage for individuals transplanted from the entrance to the inner location. The here observed results may thus indicate that the disturbance gradient in marinas might constitute a staple for selecting pollutant-resistant species and populations, causing local adaptation. This highlights the importance of conducting further studies into small scale local adaptation.

Keywords: local adaptation, fouling, pollution, metabolomics, respiration, marinas

Graphical abstract.



1. Introduction

With increasing anthropic pressure on ecosystems, understanding how communities and species respond and adapt to global change remains a major challenge. Anthropic disturbances are often intense and may overcome resistance and resilience of the local communities (Type II and III disturbances; Sutherland 1981). In natural habitats, the resilience of communities (*i.e.*, the "elasticity" of a community and its capacity to return to a stable state after disturbance) has been relatively well studied. However, resistance (*i.e.*, the capacity of communities to resist disturbance-induced changes) remains poorly studied although it is the major driver of community structure in urban habitats where disturbances do rarely relax and tend to even increase in pressure (see 'press disturbance'; Nimmo et al. 2015). As adaptation may be a major driver of resistance (Nimmo et al. 2015), understanding how eco-evolutionary feedbacks drive species adaptation in habitats subjected to anthropic disturbance seems crucial (Alberti 2015). Harbors and marinas constitute excellent study models to dive into these paradigms, as they combine multiple facets of anthropogenic disturbances (Bulleri 2006; Bulleri and Chapman 2010). Artificial structures are extremely numerous and diverse in marine urban areas where they constitute a major substratum for benthic communities (Mineur et al. 2012). Their properties are however different from natural substratum, resulting in different communities and diversity (Glasby et al. 2007; Oricchio et al. 2016; Chan and Briski 2017). In harbors, some artificial structures like

breakwaters and jetties are intended to modify/reduce hydrodynamism which may impact connectivity and local biodiversity (Floerl and Inglis 2003; Fauvelot et al. 2009). This reduction of water exchange with the open sea can also lead to an increased risk of eutrophication (Lee and Arega 1999), higher temperature fluctuations (Menniti et al. 2020), and can favor the accumulation of pollutants like Persistent Organic Pollutants (POPs) or Metallic Trace Elements (MTEs) (Owen and Sandhu 2000; Tolun et al. 2001; Schiff et al. 2007; Mohammed et al. 2011; Aly et al. 2013). These disturbances may thus affect the communities in harbors, leading to reduced biodiversity and increasing the presence of opportunistic species (Dijkstra et al. 2007; Saloni and Crowe 2015; Kinsella and Crowe 2016). The innermost parts of a marina may be the most affected by this phenomenon due to even lower water mixing (Schiff et al. 2007). Hence, as these disturbances may increase in intensity and frequency towards the inner parts of marinas, they might result in a disturbance gradient and constitute a selective filter for organisms, with gradient magnitude and orientation potentially modulated by harbor morphology (Floerl and Inglis 2003).

Harbors are a major area of species introduction as they are at the end points of main introduction vectors through maritime traffic (Mineur et al. 2008; Sylvester et al. 2011; Ferrario et al. 2017). Introduced species, if they become invasive, sometimes completely restructure ecosystems, often leading to the loss of biodiversity and ecosystem services (Pejchar and Mooney 2009; Johnston et al. 2015b; Walsh et al. 2016). Together with the cost of mitigating invasive species, this loss of services can cause severe economic impacts, ranging from hundreds of millions to tens of billions dollars, depending on the country (Lovell et al. 2006; Olson 2006; Jardine and Sanchirico 2018). Introduced species seem to be favored in disturbance regimes (Hobbs and Huenneke 1992; Altman and Whitlatch 2007; Bulleri et al. 2016) as they are often described as opportunistic and more resistant to high and drastic fluctuations. They have for instance been shown to be more resistant to extreme temperature events (Zerebecki and Sorte 2011; Kelley 2014; Marie et al. 2017; Kenworthy et al. 2018a) which are more common in harbors due to the reduced water volume and exchange with the open sea (Menniti et al. 2020). Copper, used as active component of many antifouling coatings of ships, demonstrated its

detrimental effect on the native communities (Piola and Johnston 2008; Piola et al. 2009; Osborne and Poynton 2019), and with other pollutants has been shown to favor many introduced species in harbors due to their higher resistance to them (Piola and Johnston 2006a; Dafforn et al. 2008; Osborne and Poynton 2019). The strong selective pressure exerted by copper can even lead to differential resistance among populations of the same introduced species (Piola and Johnston 2006b).

Previous studies have observed local variation of fouling (native and introduced) communities between different parts of harbors/marinas, which has been linked to pollution gradients (Je et al. 2004; Ryu et al. 2011; Kenworthy et al. 2018b). This may indicate that the selective pressure exerted by disturbances in marinas may operate at small spatial scales, structuring the community and potentially causing local adaptation at the species level. Such studies remain however putative regarding the causal link between disturbance and community composition as they show correlative results. As correlations between phenotypes and environmental conditions may be caused by phenotypic plasticity, manipulative experiments are required to expose the link between concurrent natural selection and observed variability (Kawecki and Ebert 2004; Thorpe et al. 2005). To expose the causal link between local adaptation and specific environmental factors, reciprocal transplant experiments should be conducted with one species or with entire communities (Kawecki and Ebert 2004; Angert and Schemske 2005; Chang and Marshall 2016; Sork 2018). It is then possible to reveal the presence of local adaptation, if fitness differences exist between 'locals' originating from a studied location and 'foreign' which were transplanted to this location ('locals vs foreign' criterion; Kawecki and Ebert 2004; Martin et al. 2021). Additionally, the 'home vs away' criterion can be satisfied if individuals at 'home' have higher fitness than their transplanted counterparts ('away'). This last criterion is however not a strict requirement to conclude on local adaptation (Kawecki and Ebert 2004). To occur, local adaptation requires a strong selective filter (Kawecki and Ebert 2004), such as anthropogenic disturbances. If selective pressure is strong enough to overcome the homogenizing effect of dispersion, local adaptation at smaller scales than the dispersal neighborhood of the considered species may occur (microgeographic adaptation; Richardson et al. 2014). While spatial

96 differentiation of phenotypes may result from other processes such as spatial sorting (Shine et al.
97 2011), selection within one generation (Carlson et al. 2011), or preferential settlement according to
98 phenotype (Bierne et al. 2003), local adaptation seems to be the most frequently described. Using
99 transplant experiments, some studies have been able to show local adaptation of various species,
100 whether marine or terrestrial, to urban habitats, to pollution, and for an introduced species, to
101 environmental conditions in its invaded areas (Roesijadi et al. 1984; Colautti and Lau 2015; Martin et
102 al. 2021).

103 Kenworthy et al. (2018b) correlated a significant variation in contaminants, such as copper, zinc, and
104 hydrocarbons in marina sediments, with heterogeneity in fouling communities from pillars at small
105 spatial scale (< 100 m). To show the causal effect of the spatial variation of contaminants on
106 community composition and its effect on local adaptation, we conducted a reciprocal transplant
107 experiment in the very same marina. We chose to combine the classical tools of community analysis
108 with a functional approach (community respiration, pollutant uptake and metabolomics) to investigate
109 the response of the fauna to this environmental variation as there may be a direct interaction between
110 respiration or metabolite variations with the environment (Macel et al. 2010; Jones et al. 2013;
111 Johnston et al. 2015a; Kenworthy et al. 2018a). As metabolomics constitutes a non-targeted analysis
112 of molecules, it can be used to study non-model organisms in great detail (Macel et al. 2010; Jones et
113 al. 2013). It has already been used to attempt to explain the success of certain introduced species in
114 terrestrial and marine habitats (Nylund et al. 2011; Greff et al. 2017; Utermann et al. 2020; Skubel et
115 al. 2020) and to observe how heavy metal pollution may impact different organisms (Booth et al. 2011;
116 Kwon et al. 2012; Ji et al. 2015).

117 Our aim was to identify an anthropogenic disturbance gradient, illustrated by pollution levels and
118 temperature differences, with higher values at the inner locations of the studied marina. We
119 hypothesized that community structure would correlate with this gradient and that transplanted
120 communities would resemble the communities of the location they were transplanted to, exposing a

causal link between environmental conditions and community structure. Furthermore, we expected to verify the 'locals *vs* foreign' criterion of local adaptation on the respiration and metabolome of the studied models. Understanding if local variations of environmental conditions at small scales could lead to locally distinct communities and local adaptation may be crucial for understanding the processes of contemporary evolution of introduced species (Colautti and Lau 2015); and in consequence might be crucial for understanding the process of invasion itself.

2. Materials and methods

2.1 Study site

The Marina du Château (48°22'43.4"N; 4°29'22.1"W) in Brest, France was chosen as study site. This recreational marina, nested within the highly anthropized urban area of Brest, is integrated in a 250 ha harbor including a commercial (> 2.10^6 $t.yr^{-1}$ merchants, fishing and petroleum activities) and a military (2^{nd} biggest French arsenal) part, which occupies the major part of the city coastline. The marina is characterized by numerous artificial substrata, colonized by fouling communities including several introduced species (Kenworthy et al. 2018b). We focused this study on floating pontoons as they are one of the most frequent substrata in the marina. In accordance with this previous study, the same three locations (inner, middle and entrance; spaced < 100 m) were studied.

2.2 Reciprocal transplant experiment

At each of the 3 locations, 20 black polyethylene panels (Correx, 0.2 m x 0.2 m, 3 mm thick) were deployed in April 2019 for a reciprocal transplant experiment. The panels were sufficiently spaced to be considered as independent samples. After two months in the field (60 days, June), panels were gathered and photographed. At each location, 5 panels were sampled for immediate species identification. The remaining panels were randomly assigned to either be transplanted to one of the two other locations (10 panels, 5 per location), or stayed in place as control (5 panels per location).

Consequently, over a total of 15 panels present at each location, 10 were originating from the other two locations after transplant. Each panel could thus be sorted in further analysis according to a treatment depending on its origin (*i.e.,* where recruitment started before transplant) and its destination (*i.e.,* where it was transplanted to). Henceforth, we use the following code for treatment identification: 'I' Inner, 'M' Middle, 'E' Entrance; '>' indicates the direction of transplant (e.g., I>E corresponds to panels from the inner location transplanted to the entrance E); C control (ex. IC is the inner control, *i.e.,* a treatment where panels stayed at the inner location during the whole experiment). After two additional months (70 days, September 2019) the whole set of panels was measured *in situ* for community respiration, then photographed, and fauna samples (individuals of key species) were immediately fixed in dry ice for contaminant and metabolomic assessment. The panels with the community were then transported in sea-water containers and stored in aquaria facilities (max. 1 week) for taxonomic determination on life communities.

2.3 Environmental assessment

At each location, water temperature was recorded with 1-hour interval all over the 4-month long experiment at 1 m depth (panels depth) using a HOBO® (Onset®) TidbiT v2 Water Temperature Data Logger. Here the daily average was used for further analysis. Hydrodynamism was measured with 5-min interval using a HOBO® (Onset®) Pendant G Data Logger until the battery went flat (73 days). Hydrodynamism was approximated by the daily total position change based on differences in acceleration between successive 5-min intervals which was calculated as

$$\sum_{tn}^{t0}([\vec{a}_x(t) - \vec{a}_x(t-1)] + [\vec{a}_y(t) - \vec{a}_y(t-1)] + [\vec{a}_z(t) - \vec{a}_z(t-1)])$$

where *t* is the measurement at a given time; *n* the number of measurements during a day, $\vec{a}$ the acceleration in g (9.81 m.s^{-2}) in the x, y, or z axis. As the logger data consist in time series, requiring specific statistical tests, two linear mixed effects (LME) model fit by restricted maximum likelihood

(REML) were used (explaining temperature or hydrodynamism by location and using date as random factor) with t-test with Satterthwaite's method from the 'lme4' and 'lmerTest' package in R (version 3.1-3, Zeileis and Hothorn 2002; version 1.1-26; Bates et al. 2015).

At the end of the experiment, at each location, surface sediments were sampled by divers for quantification of Metallic Trace Elements (MTEs, 5 replicates of 400 g each) and for Persistent Organic Pollutants (POPs, 3 replicates of 400 g each). Several colonies of the bryozoan *Bugula neritina* (Linnaeus, 1758), which was present at all locations all along the experiment, were sampled from each panel (5 replicates per treatment, sometimes pooled colonies) and frozen *in situ* in dry ice before storage in the lab at -80°C. Large colonies were favored as they were more likely to have recruited before transplant. Due to the abundance of this species, the sampling of colonies was assumed not to influence the community structure.

Sediment and animal MTEs samples were dissolved in a mixture of three-acids (HCl, HNO_3, HF; all Suprapur grade). The solutions were then analyzed for Metallic Trace Elements (MTE) using a Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS; ELEMENT XR, ThermoElement) as in Jacquet et al. (2021). The instrument was calibrated using standard solutions, and we used an external calibration method with Indium as an internal standard. The efficiency of the dissolution procedure and the quality of the SF-ICP-MS measurements were controlled by the determination of element concentrations on Certified Reference Materials (MESS-4, SLRS-5, SLEW-3 and CASS-5). Concentrations and the list of targeted MTEs is reported in Tab. 1.

The POPs analytical method for Polychlorinated biphenyls (PCBs) and pesticides in sediments has been described in Wafo et al. (2006). The protocol for *B. neritina* samples followed the same procedure. Our analysis focused on 33 individual PCB congeners which include PCB contamination indicators targeted by the International Council for the Exploration of the Sea (ICES) and congeners with high environmental prevalence (Webster et al. 2013). A total of 17 pesticides including lindane, DDT, aldrin were measured (list in Sup. Tab. 1). Polycyclic Aromatic Hydrocarbons (PAH) determination followed

established protocols (Sarrazin et al. 2006; Ratier et al. 2018; Dron et al. 2019) and is also available in detail in the supplementary material of Gauff et al. (2022). All 16 PAH congeners defined by the US Environmental Protection Agency (USEPA) priority list (US EPA 2014) were targeted (list in Tab. 2) and each was identified taking into account the retention time and the chromatogram mass spectrum in full scan mode.

For consistency, all contaminants were tested for differences among locations using a Kruskal-Wallis test in R (version 4.0.3; R Core Team 2020) followed by a Dunn test from the 'FSA' package (version 0.8.32; Ogle et al. 2021), as conditions for the application of parametric tests were not always satisfied. Reference values by the Canadian sediment quality guidelines (CCME 1999), by the US Environmental Protection Agency (US EPA 2005), and by the French national monitoring network of water and sediment qualities in maritime harbors (REPOM 2013) were added. For MTEs, an additional sediment quality category (SQC) by Guerra-García et al. (2021) was reported. In order to integrate all contaminants into one metric, normalized contaminant concentrations in *B. neritina* were used:

$$C_{i(norm)} = {(C_i - C_{min})}\Big/{(C_{max} - C_{min})}$$

where: $C_{i(norm)}$ is the i^{th} normalized value; C_i is the i^{th} value; C_{min} and C_{max} are the minimum and maximum values of contaminant concentrations. The normalized data was plotted in a boxplot for each transplant and control treatment with 'ggplot2' in R (version 3.3.3; Wickham 2016). A two-level ANOVA was used to test for the effect of Origin and Destination on the normalized concentration of all contaminants in *B. neritina*, as well as for the effect of their interaction. A Tukey's honest significance test (Tukey HSD; Tukey 1949) was conducted to test for differences of the normalized concentration between treatments. To visualize the importance of individual contaminants in *B. neritina* samples, we additionally conducted a Principal Component Analysis (PCA; 45 variables) with auto-scaled variables using the R package 'FactoMineR' (version 1.34; Lê et al. 2008). PCAs are a highly powerful technique for analyzing multinormal quantitative 'environmental' data (here contaminants in tissue samples; Borcard et al. 2018)

220

221 2.4 Community taxonomic assessment

222 Photography analysis of panels was used to check for community homogeneity within each location
223 before transplant. This allows to exclude experimental artifacts resulting from initial differences
224 between control and transplanted panels as well as panels sampled before transplant. The outermost
225 centimeter of the panels was excluded from our analysis to avoid an edge effect. All photos were
226 analyzed by a single observer using a 144-point stratified random-point overlay. This number of points
227 (> 0.4 points.cm^{-2}) provides a good tradeoff between efficiency and precision, providing a 0.95
228 confidence interval for species contributing at least to 5% cover (Taormina et al. 2020). Similarity of
229 panels within each location before transplant, was checked using a PERMANOVA with the R package
230 'vegan' (version 2.4-6; Oksanen et al. 2018). Confirming this homogeneity allowed to entirely focus on
231 the analysis of panels sampled before transplant (5 per location) and at the end of the experiment (15
232 per location) for which higher data quality is expected due to higher taxonomic resolution.

233 At the two studied dates (before transplant and 2 months after transplant), the sampled panels were
234 analyzed in the laboratory for taxonomic identification on living organisms. A 144-point grid was
235 superimposed on the panels (stratified point design) and every individual of each species was counted
236 at each point. This allowed to account for epibionts as well as the different strata of the community
237 and to give more detail than a photographic (2-D) analysis. Species were identified to the lowest
238 possible taxonomic level (Hayward and Ryland 1979, 1985, 1995, 1998; Brunetti and Mastrototaro
239 2017). All community data were explored with the R package 'vegan' (version 2.4-6; Oksanen et al.
240 2018) and analyzed according to recommended workflows applicable for this kind of data (Borcard et
241 al. 2018). The multi-strata community matrix was transformed into a Bray-Curtis dissimilarity matrix.
242 First, we analyzed the panels sampled before transplant. Upon confirming homogeneity of group
243 dispersions with the 'betadisper' function, a PERMANOVA (10^4 permutations) was conducted, followed
244 by a pairwise PERMANOVA from the 'pairwisAadonis' R package (version 0.3; Martinez Arbizu 2019)

245 including a Benjamini-Hochberg correction (Benjamini and Hochberg 1995). For all sampled panels, the
246 mean species richness, Shannon diversity index and Pielou's evenness were calculated and the mean
247 cover of the three most abundant species was assessed for each treatment. A PERMANOVA (10^4
248 permutations) testing for the effect of origin and destination of panels as well as their interaction was
249 conducted for panels at the end of the experiment. For the factor 'Origin' as well as the interaction of
250 Origin and Destination (*i.e.*, Treatment), homogeneity of group dispersions was compromised.
251 However PERMANOVA shows high robustness in balanced designs such as here (Anderson and Walsh
252 2013). A pairwise PERMANOVA (10^4 permutations) including a Benjamini-Hochberg correction was
253 conducted to test for differences of communities between treatments. The results were visualized
254 using a Principal Coordinates Analysis (PCoA). The 'envfit' function was used to overlay species vectors,
255 which were significantly correlated to the ordination (with $p < 0.05$ and $R^2 > 0.2$). To identify the
256 indicator species in our experiment, we used a multipattern analysis from the 'indicspecies' R package
257 (version 1.7.9; Cáceres et al. 2011). Parameters were set to 10^4 permutations while testing for
258 individual treatments, allowing combinations of treatments within the same origin or within the same
259 destination (ex.: origin inner = IC + I>M + I>E).

260

261 ## 2.5 Respiration measures

262 Community respiration was measured *in situ* for all panels at the end of the experiment. Panels were
263 detached from the pontoons and individually hooked in 23.3 L transparent, hermetically sealed
264 methacrylate chambers. A magnetic stirrer was used to ensure water circulation within the chamber.
265 Chambers were placed under the pontoons for similar light conditions as during the experiment. Three
266 empty control chambers (blank) were incubated at each location to control for potential
267 photosynthesis or respiration caused by microbial and plankton activity within the water. Dissolved
268 oxygen concentration was measured extracting 200 mL of water from a valve on the chamber using a
269 syringe and with an oxygen probe with temperature sensor (Hach-Lange LDO101). Oxygen

concentration was measured right after sealing off the chamber and at the end of incubation (between 14 and 23 min). The measured ΔO_2 (in mg $O_2.L^{-1}$) was then rated according to the volume of the chamber, the incubation time, and the ash free dry mass (AFDM) to obtain the respiration in mg $O_2.h^{-1}.g_{AFDM}^{-1}$. The AFDM was determined after the community analyses in the laboratory were finished. The whole community was dried for one week at 60°C for each panel. After a first mass measure, it was burned at 520°C for 6 h to allow to calculate the AFDM. For respiration, two aberrant values were excluded from analysis. Respiration measurements were compared between treatments *via* an ANOVA followed by a Tukey HSD test.

2.6 Metabolome assessment

Three species occurring at the three locations in the marina were sampled for metabolomic analyses: two large colonies of the bryozoan *Bugula neritina* per panel and one individual per panel of the two solitary ascidians *Ciona intestinalis* (Linnaeus, 1767) and *Ascidiella aspersa* (Müller, 1776). For *B. neritina* and *C. intestinalis*, only four samples could be constituted for the E>I. Additionally, the IC and M>I treatment are missing entirely for *C. intestinalis* due to the absence of individuals for these conditions. After sampling on the field, samples were immediately frozen in dry ice and then stored at -80°C. Samples were analyzed according to different mass spectrometry approaches. The detailed extraction, injection protocols and Mass Spectrometry (MS) modalities (non-fragmenting modes $LCMS^{1+}$, $LCMS^{1-}$; Fragmenting modes $LCMS^{2+}$, $LCMS^{2-}$; and GCMS) can be consulted in the supplementary material. For each species, all features from the $LCMS^{1+}$, the $LCMS^{1-}$ as well as the GCMS were combined into one matrix and treated with 'Metaboanalyst' (version 5.0; Xia et al. 2009). The imported matrix (with less than 5000 features) was log transformed and auto scaled. We conducted a Partial Least Squares Discriminant Analysis (PLS-DA) with class order assuming a gradient from the inner to the entrance location, providing the best discrimination among treatments (Xia et al. 2009; Kuhlisch and Pohnert 2015). In parallel a PPLS-DA driven permutation Model Validation Analysis (MVA)

as well as a pairwise MVA test using the 'RVAideMemoire' package in R (version 0.9-79; Hervé 2021) were performed to test for statistical differences between treatments (10^4 permutations, 6 components). After revealing significant differences between treatments for *B. neritina*, the 100 first Variable Important in Projection (VIP) scores were selected and tentatively annotated with the help of the $LCMS^2$ (fragmentation) data. The annotated VIP were gathered in a table including an expression heatmap ranging from the highest to the lowest ion intensity in $LCMS^1$ for each treatment. Mean intensity was used when a feature was detected as a VIP in $LCMS^{1+}$ and $LCMS^{1-}$. Annotated metabolites were sorted following their heatmap similarity in the dendrogram, while allowing compromise for keeping chemical families together for clarity. Molecules within a family were sorted by numbers of carbons, oxygens, and unsaturations. For *C. intestinalis* a more streamlined approach was used for simplification as some treatments were missing. Using a molecular network generated on Global Natural Products Social Molecular Networking (GNPS; version 1.3.15; Wang et al. 2016) and visualized on 'cytoscape' (version 3.7.2), we localized clusters in $LCMS^{2+}$ and $LCMS^{2-}$ corresponding to glycerophospholipids and only highlighted VIP within these clusters.

3. **Results**

3.1 Environmental characterization

Significantly higher hydrodynamism indicating higher water exchanges with external mass waters was noted at the entrance location (LME; $t = 3.61$; $p < 0.001$; Sup. Fig. 1). Temperature did also significantly vary between locations (LME; $t = 5.33$; $p < 0.05$; Sup. Fig. 1). However, these differences (< 0.1°C) are well below temperatures that are expected to have an impact on fauna (Brunetti et al. 1980; Hitoshi and Kazutsugu 1984; Qiu and Qian 1998; Sorte et al. 2011; Lord 2017).

Almost all MTEs were distributed along a decreasing concentration gradient from the inner location to the entrance, although differences were not always supported by statistics. Only Cu, Pb and Zn showed

significantly different concentrations between locations (Kruskal-Wallis tests; $\chi^2 > 8.07$; $p < 0.05$; Tab. 1), with Cu and Zn significantly higher at the inner location (Dunn Test; $Z > 2.83$; $p.adj < 0.05$). When applicable, most MTE concentrations are barely above the Canadian sediment quality guideline (CCME) and correspond to moderate to good Sediment Quality Categories for Cu and Pb according to Guerra-García et al. (2021; Tab. 1). For Cu this constitutes an increase compared to 2011 and 2012, when concentrations were below REPOM category N1 (45 $mg.kg^{-1}$; REPOM 2013), while they were above in the present study.

Seven pesticides, two PAHs, as well as total PCB (tPCB), also showed significant differences in concentration in sediments between locations (Kruskal-Wallis test; $\chi^2 > 6.47$; $p < 0.05$; Sup. Tab. 1; Tab. 2). All tested PAHs exceed CCME guidelines and US-EPA concentrations for which 20% of sediments may be toxic to model amphipods (Tab. 2; CCME 1999; US EPA 2005). REPOM classification for PAHs was constant between 2012 and 2019 (REPOM 2013), and a slight improvement of the contamination in Benzo[*a*]pyrene decreasing below N1 can be noted. The PAHs chrysene, fluoranthene, fluorene and phenanthrene largely exceeded values at which adverse effects on fauna may be encountered (Tab. 3; CCME 1999; US EPA 2005). Total PCBs (tPCB) and two pesticides (Lindane and pp'-DDD) were also more concentrated than recommended (Sup. Tab. 1; Tab. 2; CCME 1999).

Tab. 1: Mean values (mg.kg^{-1} = ppm) with standard deviation of the Metallic Trace Element (MTE) concentrations in the sediments of the three locations (n = 5) sampled at the end of the experiment and in the bryozoan *Bugula neritina* (control individuals; n = 5). Stars indicate significant differences between locations (Kruskal-Wallis test; *: p < 0.05; **: p < 0.01). Sediment quality guidelines provided by the Canadian Council of Ministers of the Environment (CCME 1999; ISQG: Interim Sediment Quality Guideline; PEL: Probable Effect Level); the United Stated Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples); the Réseau de surveillance des ports maritimes (REPOM 2013, Tab. 4; N1 level of contamination requiring further assessment; N2 level of contamination with probable negative impact); and Sediment Quality category (SQC) by Guerra-García et al. (2021), are indicated. Bold font for reference values: threshold exceeded at least at one location.

Metallic Trace Elements (mg.kg^{-1})		Location (mean ± SD)			K-W test	CCME		US EPA		REPOM		SQC
		Inner	Middle	Entrance		ISQG	PEL	T20	T50	N1	N2	
^{27}Al	Sediment	29207 ± 3825	26647 ± 9596	23125 ± 12905	ns							
	B. neritina	416 ± 142	246 ± 85	123 ± 36	**							
^{75}As	Sediment	14.4 ± 3.2	11.3 ± 2.9	8.77 ± 5.1	ns	**7.24**	41.6	**7.4**	20	25	50	Good
	B. neritina	3.46 ± 0.9	3.51 ± 0.8	2.56 ± 0.1	ns							
^{59}Co	Sediment	5.78 ± 0.6	5.5 ± 1.2	4.72 ± 2.7	ns							Good
	B. neritina	0.2 ± 0.1	0.18 ± 0.1	0.07 ± 0.03	*							
^{52}Cr	Sediment	57.4 ± 7.3	50.7 ± 11	43.6 ± 25	ns	**52.3**	160	**49**	140	90	180	Good
	B. neritina	0.96 ± 0.1	0.64 ± 0.2	0.29 ± 0.1	**							
^{63}Cu	Sediment	83.9 ± 14	45 ± 10	32.8 ± 19	**	**18.7**	108	**32**	94	**45**	90	**Moderate**
	B. neritina	3.08 ± 3.5	1.1 ± 1.2	4.61 ± 5.7	ns							
^{56}Fe	Sediment	13569 ± 1427	12639 ± 2980	10643 ± 6037	ns							
	B. neritina	521 ± 105	408 ± 138	172 ± 56	*							
^{55}Mn	Sediment	150 ± 22	154 ± 29	134 ± 74	ns							
	B. neritina	22.6 ± 5.1	20.2 ± 10	8.1 ± 3.3	*							
^{60}Ni	Sediment	18.1 ± 1.8	16.6 ± 3.8	14.3 ± 8.2	ns			**15**	47	37	74	Good
	B. neritina	0.72 ± 0.2	0.54 ± 0.2	0.27 ± 0.1	**							
^{208}Pb	Sediment	85 ± 12	48.2 ± 14	45.8 ± 25	*	**30.2**	112	**30**	94	100	200	**Moderate**
	B. neritina	5.3 ± 2.3	2.71 ± 0.5	1.56 ± 0.4	**							
^{47}Ti	Sediment	2283 ± 291	2285 ± 506	1923 ± 1094	ns							
	B. neritina	6.15 ± 4	3.44 ± 1.5	1.53 ± 0.7	*							
^{51}V	Sediment	89.8 ± 11	75.6 ± 19	64.6 ± 37	ns							
	B. neritina	2.84 ± 1.4	2.53 ± 0.7	1.23 ± 0.3	*							
^{66}Zn	Sediment	236 ± 89	178 ± 77	111 ± 60	*	**124**	271	**94**	240	276	552	Good
	B. neritina	58.5 ± 16	53.5 ± 13	31.9 ± 4.2	*							

Tab. 2: Mean values (µg.kg^{-1} = ppb) with standard deviation of Polycyclic Aromatic Hydrocarbons (PAH) and Total Polychlorinated Biphenyl (tPCB) concentrations in the sediments (n = 3) and in the bryozoan *Bugula neritina* control individuals (n = 5) of the three locations. Stars indicate significant differences between locations (Kruskal-Wallis test; *: $p < 0.05$). Sediment quality guidelines provided by the Canadian Council of Ministers of the Environment (CCME 1999; ISQG: Interim Sediment Quality Guideline; PEL: Probable Effect Level); the United Stated Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples); and the Réseau de surveillance des ports maritimes (REPOM 2013, Tab. 5 and 6; N1 level of contamination requiring further assessment; N2 level of contamination with probable negative impact) are indicated. Bold font for reference values: threshold exceeded at least at one location.

PAH (µg.kg^{-1})		**Location mean ± SD**			**K-W**	**CCME**		**US EPA**		**REPOM**	
		Inner	**Middle**	**Entrance**	**test**	**ISQG**	**PEL**	**T20**	**T50**	**N1**	**N2**
Acenaphthene	Sediment	39 ± 8.5	34.1 ± 2	33.6 ± 1	ns	**6.71**	88.9	**19**	120	**15**	260
	B. neritina	1.97 ± 1.2	2.09 ± 0.1	0.88 ± 0.1	ns						
Acenaphthylene	Sediment	50.6 ± 17	65.7 ± 8.3	60.6 ± 22	ns	**5.87**	128	**14**	140	**40**	340
	B. neritina	2.81 ± 0.7	2.26 ± 0.1	1.21 ± 0.2	ns						
Anthracene	Sediment	58.6 ± 7.9	52.1 ± 12	44.6 ± 14	ns	**46.9**	245	**34**	290	85	590
	B. neritina	5.3 ± 0.2	5 ± 0.1	1.87 ± 0.1	ns						
Benzo[*a*]anthracene	Sediment	379 ± 23	343 ± 15	354 ± 1.7	ns	**74.8**	693	**61**	470	**260**	930
	B. neritina	8.43 ± 0.5	2.46 ± 0.1	6.1 ± 0.8	ns						
Benzo[*a*]pyrene	Sediment	324 ± 69	425 ± 109	407 ± 43	ns	**88.8**	763	**69**	520	430	1015
	B. neritina	9.93 ± 0.7	8.9 ± 0.3	7.4 ± 0.8	ns						
Benzo[*b*]fluoranthene	Sediment	700 ± 67	651 ± 141	880 ± 232	ns			**130**	1110	**400**	900
	B. neritina	23.5 ± 2.3	26.8 ± 3.8	21.4 ± 1.2	ns						
Benzo[*g,h,i*]perylene	Sediment	407 ± 79	212 ± 51	283 ± 7.9	*			**67**	500	1700	5650
	B. neritina	10 ± 0.3	7.95 ± 0.3	10 ± 0.1	ns						
Benzo[*k*]fluoranthene	Sediment	328 ± 72	413 ± 48	537 ± 78	ns			**70**	540	**200**	**400**
	B. neritina	26.5 ± 4.1	13.4 ± 0.2	13.1 ± 0.2	ns						
Chrysene	Sediment	943 ± 185	1051 ± 61	1192 ± 173	ns	**108**	**846**	**82**	**650**	**380**	1590
	B. neritina	21.5 ± 1.3	24.8 ± 2	15.7 ± 1	ns						
Dibenzo[*a,h*]anthracene	Sediment	83.6 ± 19	69.9 ± 20	76.9 ± 19	ns	**6.22**	135	**19**	110	**60**	160
	B. neritina	4.73 ± 0.5	3.94 ± 0.1	2.85 ± 0.3	ns						
Fluoranthene	Sediment	1532 ± 430	1049 ± 71	1829 ± 268	ns	**113**	**1494**	**120**	**1030**	**600**	2850
	B. neritina	43.4 ± 1.6	33.9 ± 2.6	15.5 ± 0.1	ns						
Fluorene	Sediment	725 ± 19	567 ± 43	642 ± 56	*	**21.2**	**144**	**19**	**110**	**20**	**280**
	B. neritina	3.42 ± 0.1	2.58 ± 0.2	1.28 ± 0.1	ns						
Indeno[1,2,3-cd]pyrene	Sediment	701 ± 106	713 ± 302	843 ± 313	ns			**68**	**490**	1700	5650
	B. neritina	14 ± 1.4	11.6 ± 0.4	12.5 ± 0.9	ns						
Naphthalene	Sediment	232 ± 32	239 ± 37	344 ± 103	ns	**34.6**	391	**30**	**220**	**160**	1130
	B. neritina	17 ± 2.7	12.5 ± 0.3	7.23 ± 0.3	ns						
Phenanthrene	Sediment	1629 ± 363	1154 ± 66	1996 ± 239	ns	**86.7**	**544**	**68**	**460**	**240**	**870**
	B. neritina	12.6 ± 0.5	44.9 ± 3.9	26.3 ± 3	ns						
Pyrene	Sediment	545 ± 7.8	510 ± 23	556 ± 17	ns	**153**	1398	**120**	930	**500**	1500
	B. neritina	10.8 ± 0.7	9.58 ± 0.6	5.66 ± 0.6	ns						
PCB (µg.kg^{-1})		**Location mean ± SD**			**K-W**	**CCME**		**US EPA**		**REPOM**	
		Inner	**Middle**	**Entrance**	**test**	**ISQG**	**PEL**	**T20**	**T50**	**N1**	**N2**
tPCB	Sediment	603 ± 5,9	506 ± 12	544 ± 2,5	*	**21.5**	**189**	**35**	**370**	**500**	1000
	B. neritina	360 ± 13	216 ± 10	155 ± 7.1	*						

For control (*i. e.* not transplanted) individuals of *B. neritina* sampled at the end of the experiment, all MTEs excepted Cu and As showed significant differences between the three locations, with systematically higher concentrations from individuals sampled at the inner location (Kruskal-Wallis test; $\chi^2 > 6.76$; $p < 0.05$; Tab. 1). tPCB and eight pesticides did vary with distinct distribution profiles (Kruskal-Wallis test; $\chi^2 > 7.2$; $p < 0.05$; Tab. 2; Sup. Tab. 1). None of the PAHs significantly varied in *B. neritina* (Tab. 2).

The normalized concentrations of all combined contaminants varied significantly between treatments for *B. neritina* with a significant effect of 'origin' (two-way ANOVA; $p < 0.001$; $F = 7.23$), 'destination' (two-way ANOVA; $p < 0.001$; $F = 77.86$) and their interaction (*i.e.* treatment; two-way ANOVA; $p < 0.001$; $F = 9.70$). The Tukey HSD test revealed that contaminant concentrations for the inner control IC had significantly higher values than the two other controls ($p < 0.01$; Fig. 1). Higher contaminant concentrations in *B. neritina* samples transplanted to the inner location were systematically observed, compared to samples present at the entrance location at the end of the experiment, regardless of their origin ($p < 0.001$; Fig. 1). It was only possible to observe an effect of origin for samples transplanted to the inner location: E>I individuals had significantly higher normalized contaminant concentrations than M>I and IC ($p < 0.01$; Fig. 1).

The PCA biplot revealed differences of contaminant compositions of *B. neritina* between the treatments (Sup. Fig. 2). The PC1 axis (30.2%) represents general pollution levels with MTE, PCBs, Pesticides (group 2, see Sup. Fig. 2 legend) and HAP being correlated to the axis, and Phenantrene and pesticides (group 1, see Sup. Fig. 2 legend) being anticorrelated. Individuals transplanted from other locations to the inner, most notably E>I, were correlated to PC1 indicating high levels of contaminants in their tissues. Individuals which had the entrance as their destination were anticorrelated with MTE, PCBs, pesticides (group 2) and HAP and correlated with pesticides (group 1) and Phenantrene. On the PC2 (21%) MTE, PCBs and Pestistices distinguish from HAP pollutants forming two large vector groups.

Most individuals were however in the center of both PC, indicating intermediate levels for all contaminant groups.

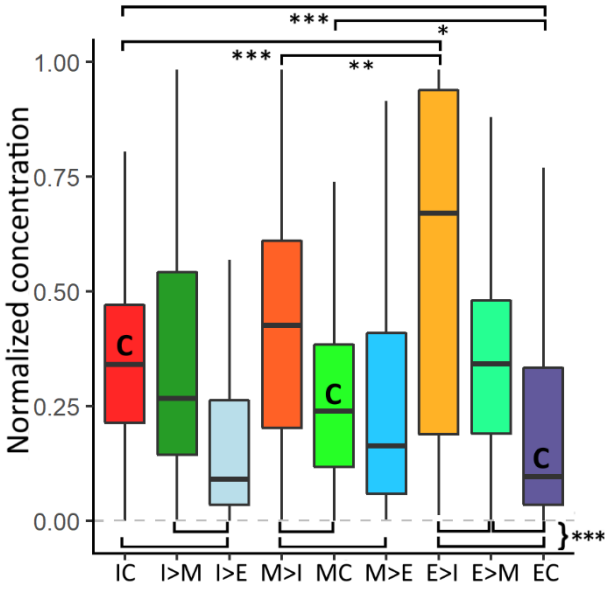


Fig. 1: Boxplot of normalized concentrations of all contaminants in the bryozoan *Bugula neritina* according to their treatment. Treatments are labelled according to their origin followed (>) by their destination 2 months after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated according to their destination (red = inner; green = middle; blue = entrance). Significant pairwise differences (Tukey's honest significance test of a 2-way Anova) are indicated by black brackets. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; no bracket: non-significant. All bottom brackets are ***. (n = 5)

3.2 Community

The photography-based community analysis did not show any difference among panels within each location before transplant (PERMANOVA; $p > 0.57$; $R^2 = 0.001$), thus excluding experimental artifacts due to panel selection. Panels sampled before transplant were significantly different according to their origin: IC vs MC (pairwise PERMANOVA; $p = 0.009$; $R^2 = 0.55$), MC vs EC (pairwise PERMANOVA; $p = 0.009$; $R^2 = 0.87$), IC vs EC (pairwise PERMANOVA; $p = 0.009$; $R^2 = 0.89$). The PCoA (Fig. 2a) clearly discriminated EC from IC and MC along the first PCoA axis (76.6% of variability) while the second PCoA axis discriminated the two latter (13.2% of variability). The cover of several species varied between treatments before transplant. *C. intestinalis*, *A. aspersa* and *B. neritina* had the highest cover at the entrance location, while *W. subatra* had a lower cover at this location and higher at the inner location

(Dunn Test; $p < 0.05$; Sup. Fig. 3). Bare space was the highest at the inner location compared to the entrance (Dunn Test; $p = 0.002$). The total cover of introduced species was higher at the entrance location than at the inner location (Dunn Test; $p = 0.012$).

Two months after transplant, the effect of 'origin' and 'destination' of panels as well as their interaction was tested and showed a significant effect of all three (PERMANOVA; $p < 0.001$; $R^2 > 0.22$). The PCoA explained a total of 79.3% of the variability (Fig. 2b), with the first axis (56.6%) associated with the destination effect (inner locations on the right side). Most species correlated positively along this axis ($p < 0.05$; $R^2 > 0.2$) are encrusting species including NIS like *Cryptosulla pallasiana* (Moll, 1803) and *Watersipora subatra*. The second axis (22.7% of variability) reflects an origin effect (all panels originating from the entrance are on the top side). E>M treatment were very distinct from all other treatments in relation with *C. intestinalis* and *Diplosoma listerianum* (Milne Edwards, 1841). Subsequent pairwise PERMANOVA for panels after the 2-months transplant revealed significant differences between almost all treatments ($p < 0.05$; $R^2 > 0.23$). Mean species richness (S), Shannon index (H') and Pielou's evenness (J') varied among sites (Sup. Tab. 2). The most abundant species (cover > 30%) were *Watersipora subatra* (Ortmann, 1890), *B. neritina* and *C. intestinalis*. Their cover was highly variable depending on the treatment (Sup. Tab. 2 and Sup. Fig. 3). Diversity indices for each treatment varied between treatments, ranging from 14.6 to 18.4 for mean species richness, from 2.0 to 2.9 for Shannon index and from 0.52 to 0.70 for Pielou's evenness. The only exception were E>M panels with very low diversity ($S = 11$; $H' = 0.6$; $J' = 0.19$; Sup. Tab. 2). *W. subatra* had a higher cover at the inner location compared to the entrance (Dunn Test; $p = 0.01$). Further details on the cover of each can be seen in Sup. Fig. 3.

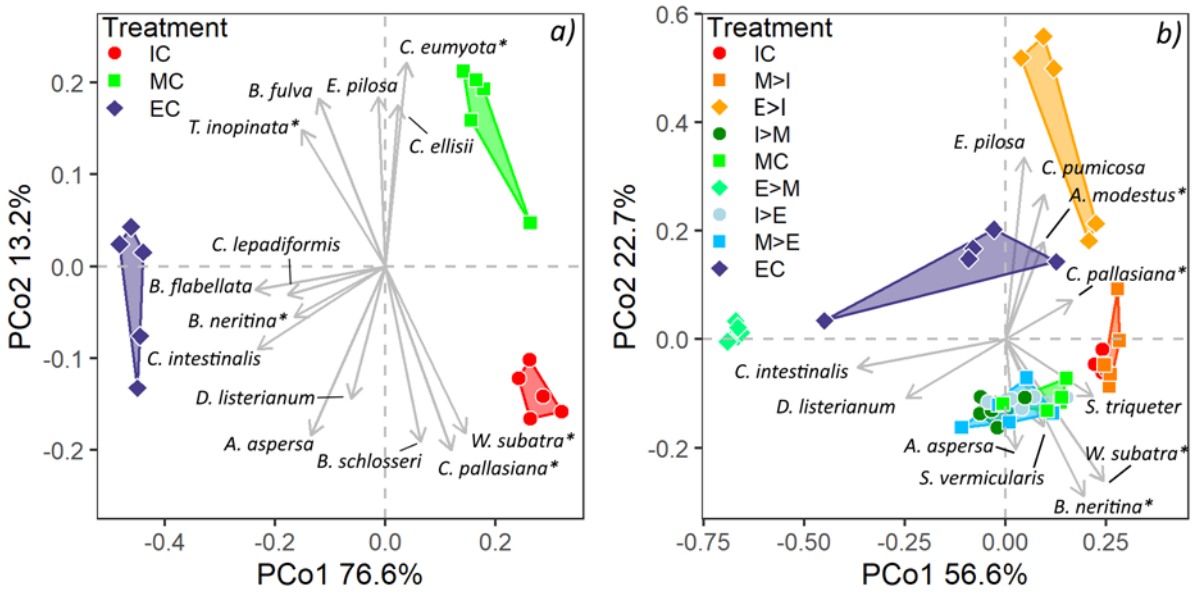


Fig. 2: Principal Coordinate Analysis (PCoA) biplot of the community structure before transplant (a) and 2 months after transplant (b). Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated in the biplot according to their destination (red = inner; green = middle; blue = entrance) and origin (circle= inner, square = middle, diamond = entrance). Vector overlay indicates species with a positive correlation ($p < 0.05$; $R^2 > 0.2$) with groups. Non-Indigenous species are indicated by an asterisk. Species list: *Ascidiella aspersa*, *Austrominius modestus**, *Botryllus schlosseri*, *Bugula neritina**, *Bugulina flabellata*, *Bugulina fulva*, *Ciona intestinalis*, *Cellepora pumicosa*, *Clavelina lepadiformis*, *Corella eumyota**, *Cradoscrupocellaria ellisii*, *Cryptosula pallasiana**, *Diplosoma listerianum*, *Electra pilosa*, *Tricellaria inopinata**

The multipattern analysis identified several species associated to the three locations before transplant as well as multiple species according to specific origins (origin effect) or to specific destinations (destination effect; Tab. 3). Over both periods, between 40% and 45% of these indicator species were NIS. While NIS were associated to every location before transplant, no NIS was associated to panels having the entrance as origin or destination after transplant.

Tab. 3: Indicator species associated to treatments identified by multipattern analysis before and 2-months after transplant. Association statistic (stat.) of a species with a group and p value of the multipattern analysis as well as Non-indigenous species (NIS) are indicated. Groups are labelled according to their origin or destination, composed by the three treatments within the respective origin or destination (ex: Origin inner = Inner Control, Inner>Middle and Inner>Entrance; where > represents the direction of transplant).

Group	Indicator Species	Authority	NIS	Stat.	p value	
Before transplant						
Inner	*Cryptosula pallasiana*	(Moll, 1803)	NIS	0.87	< 0.001	***
	Botryllus schlosseri	(Pallas, 1766)		0.78	< 0.001	***
	Watersipora subatra	(Ortmann, 1890)	NIS	0.74	< 0.001	***
	Austrominius modestus	(Darwin, 1854)	NIS	0.72	0.041	*
Middle	*Corella eumyota*	Traustedt, 1882	NIS	1.00	0.001	***
	Electra pilosa	(Linnaeus, 1767)		0.74	0.047	*
Entrance	*Ciona intestinalis*	(Linnaeus, 1767)		1.00	0.001	**
	Bugulina flabellata	(Thompson in Gray, 1848)		0.98	0.001	**
	Tricellaria inopinata	d'Hondt & Occhipinti Ambrogi, 1985	NIS	0.87	0.01	**
	Clavelina lepadiformis	(Müller, 1776)		0.83	0.03	*
	Ascidiella aspersa	(Müller, 1776)		0.81	0.003	**
	Bugulina fulva	(Ryland, 1960)		0.80	0.014	*
	Bugula neritina	(Linnaeus, 1758)	NIS	0.65	0.005	**
After transplant						
Origin Inner	*Asterocarpa humilis*	(Heller, 1878)	NIS	0.91	< 0.001	***
	Ascidiella aspersa	(Müller, 1776)		0.79	< 0.001	***
	Serpula vermicularis	Linnaeus, 1767		0.72	0.002	**
	Styela clava	Herdman, 1881	NIS	0.71	0.026	*
	Mytilus edulis	Linnaeus, 1758		0.63	0.023	*
Origin Middle	*Watersipora subatra*	(Ortmann, 1890)	NIS	0.71	< 0.001	***
	Bugula neritina	(Linnaeus, 1758)	NIS	0.66	0.008	**
Origin Entrance	*Electra pilosa*	(Linnaeus, 1767)		0.82	< 0.001	***
Destination inner	*Spirobranchus triqueter*	(Linnaeus, 1758)		0.81	< 0.001	***
	Cryptosula pallasiana	(Moll, 1803)	NIS	0.73	0.005	**
	Cellepora pumicosa	(Pallas, 1766)		0.71	0.009	**
	Austrominius modestus	(Darwin, 1854)	NIS	0.69	0.015	*
Destination Middle	*Ciona intestinalis*	(Linnaeus, 1767)		0.86	< 0.001	***
	Diplosoma listerianum	(Milne Edwards, 1841)		0.68	0.026	*
Entrance Control	*Laomedea flexuosa*	Alder, 1857		0.63	0.038	*

3.3 Community respiration

Community respiration varied considerably among treatments with panels having the inner location as destination (including IC) showing 3 to 5 times higher respiration rates than any other treatments (Fig. 3). Respiration was significantly different between IC (17.66 mg $O_2.h^{-1}.g_{AFDM}{}^{-1}$) and the two other controls (MC: 3.39 mg $O_2.h^{-1}.g_{AFDM}{}^{-1}$; EC: 4.83 mg $O_2.h^{-1}.g_{AFDM}{}^{-1}$; Tukey HSD; $p < 0.001$). M>I and E>I had each higher respiration rates than treatments in other destinations (Tukey HSD; $p < 0.001$). However, E>I respiration was even higher than the IC (E>I: 21.40 mg $O_2.h^{-1}.g_{AFDM}{}^{-1}$; Tukey HSD; $p = 0.033$).

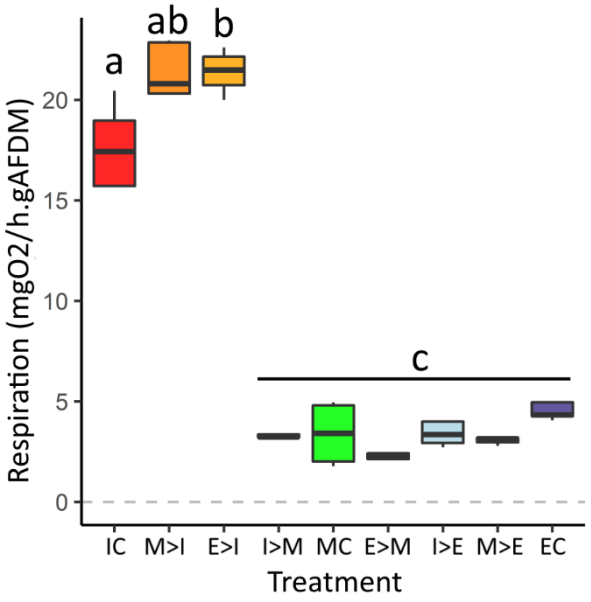


Fig. 3: Boxplot of the community respiration (mg $O_2.h^{-1}.g_{(AFDM)}{}^{-1}$) for all treatments. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated according to their destination (red = inner; green = middle; blue = entrance). Significant differences (Tukey HSD; $p < 0.05$) indicated by letter groups (n = 5).

3.4 Metabolome

The merged data matrix groups 597 features ($LCMS^{1+}$: 303, $LCMS^{1-}$: 282 and GCMS: 12) after metabolome analysis for *B. neritina*, 522 features ($LCMS^{1+}$: 181, $LCMS^{1-}$: 344 and GCMS: 7) for *C. intestinalis* and 428 features ($LCMS^{1+}$: 165, $LCMS^{1-}$: 171 and GCMS: 92) for *A. aspersa*. The PLS-DA of the detected metabolome ($LCMS^{1+}$, $LCMS^{1-}$ and GCMS) of the three studied species was established

(Fig. 4; Sup. Fig. 4). For *B. neritina* and *C. intestinalis* the treatments are well separated on the Component 1 axis, grouped by destination with the inner origin at the left side and the entrance origin on the right for each destination group. Component 2 reflects the variability within each treatment. The PPLS-DA driven permutation Model Validation Analysis (MVA) revealed significant differences among locations after transplant in the metabolomes of *B. neritina* (CER 'Classification Error Rate' = 0.53; $p < 0.001$; Fig. 4a) and of *C. intestinalis* (CER = 0.44; $p < 0.001$; Fig. 4c), but not of *A. aspersa* (CER = 0.82; $p = 0.095$; Sup. Fig. 4). The pairwise MVA test showed a strong effect on the metabolome of *B. neritina* with differences according to the treatments (Fig. 4b). The IC metabolome was significantly different from the two other controls (MVA; $p < 0.05$). Several times, transplant resulted in significantly different metabolomes compared to origin controls (destination effect) but also to destination controls (origin effect). For *C. intestinalis*, it was not possible to recover samples from the IC and M>I panels due to low biomass. Only the metabolome of E>I panels was significantly different from all other treatments, except from M>I and MC ($p < 0.047$).

For *B. neritina* the 100 highest Variable of Importance in Projection (VIP) scores linked to the PLS-DA were selected to putatively identify the metabolites linked to metabolome differentiation according to treatments. Almost two third (59 %) of the VIPs were either sulfated or phosphated compounds. Using $LCMS^2$ spectra to annotate metabolites, 46 of the 100 VIPs could be connected to a specific chemical family (Tab. 4), including 16 glycerophosphoethanolamines (35%) and 13 sulfoethanolamines (28%, a yet undescribed lipid class). While sulfoethanolamine VIPs had varying expression profiles, associated to the inner or the entrance destinations, glycerophosphoethanolamine VIPs were more prevalent in the MC and treatments including the entrance as a destination. Among other families, we were also able to annotate some oxylipins, amines and purines. For *C. intestinalis* only some glycerophospholipids were highlighted due to the absence of IC (Sup. Tab. 3). However, their pattern was similar to glycerophosphoethanolamines in *B. neritina*.

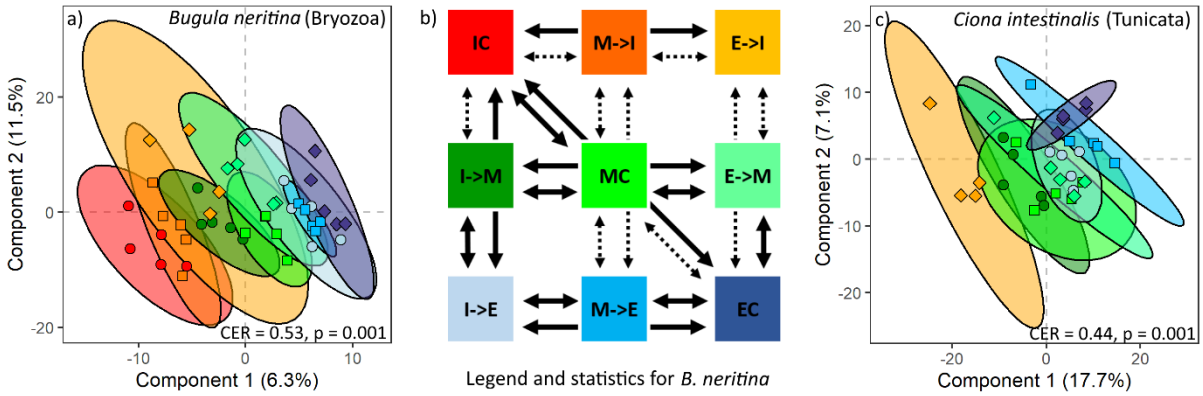
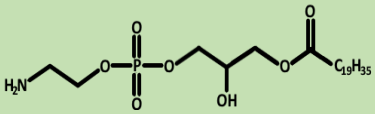
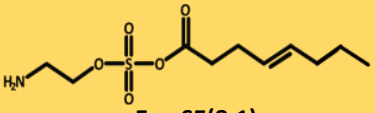


Fig. 4: a) Partial Least Squares Discriminant Analysis (PLS-DA) of the metabolomes of *Bugula neritina* with b) its associated Statistical metrics for the pairwise comparisons and c) PLS-DA of the metabolome of *Ciona intestinalis*. Ellipses indicate confidence intervals of 0.95 for each treatment. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated in the PLS-DA according to their destination (red = inner; green = middle; blue = entrance) and origin (circle= inner, square = middle, diamond = entrance). Full line arrow: pairwise MVA with $p < 0.05$. Dashed line arrow: non-significant. For both species one sample is missing in E>I while the treatments IC and M>I are completely absent for *C. intestinalis*. CER = Classification Error Rate with p-value after permanova test (permutations 10^4).

Tab. 4: Variables of Importance in Projection (VIP) scores associated to the PLS-DA of *Bugula neritina* metabolomes. 46 of 100 VIP were annotated to at least family level. Heatmap expression profiles (Prof.) were sorted while keeping chemical families together. Molecules within a family are sorted with increasing number of carbons, number of oxygens, and number of unsaturations. The two most prominent molecular families are in bold. Phosphated molecules are hued in green, sulphated molecules in yellow, oxylipins in grey and purines in blue. Treatments are labelled in the heatmap according to their origin (top) followed by their destination (below) after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination). Heatmap legend above heatmap. Asterisk on SE (Sulfoethanolamine) indicates an Acyl-SE rather than a Alkyl-SE (no asterisk).

Prof.	Chemical family	Molecular formula	Putative molecular structure	Heatmap (Low – High)
Inner Destination	Amine	$C_8H_{11}N$	Phenylethylamine	
		$C_{10}H_{12}N_2$	Tryptamine	
	Carnitine	$C_{12}H_{23}NO_4$	Valerylcarnitine	
	Glycerophosphoethanolamine	**$C_{23}H_{46}NO_6P$**		
	Glycerophosphocholine	$C_{28}H_{48}NO_7P$	PC(20:5)	
	Fatty Alcohol Sulfate	$C_{10}H_{20}O_4S$	Decenol, hydrogen sulfate	
	Sulfoethanolamine	**$C_{10}H_{21}NO_4S$**	**SE*(8:1)**	
		$C_{12}H_{34}N_2O_5S$		
		$C_{14}H_{36}N_2O_5S$		
		$C_{22}H_{41}NO_6S$	**SE(17:3)**	
Middle Destination	Brominated imidazole	$C_3H_1Br_3N_2$	1H-Imidazole, 2,4,5-tribromo-	
	Purine	$C_{11}H_{14}N_6O_4$	Adenosine, amino-	
	Glycerophosphoethanolamine	**$C_{21}H_{44}NO_6P$**	**LysoPE(P-16:0)**	
	Sulfoethanolamine	**$C_{12}H_{21}NO_4S$**		
	Sulfo serine ester	$C_{16}H_{33}NO_5S$	Serine, docosyl ester, hydrogen sulfate (ester)	
	Sulfated acid	$C_8H_8O_7S$	Sulf. benzoic/benzenacetic acid	
	Oxylipins	$C_{18}H_{32}O_7$		
		$C_{20}H_{34}O_8$		
		$C_{20}H_{34}O_8$		
	Fatty acyl glycerol	$C_{21}H_{34}O_4$	1-Oxo-octadecatetraenyl-glycerol	
Middle Control and Entrance Destination	**Glycerophosphoethanolamine**	**$C_{22}H_{44}NO_7P$**	**LysoPE(17:1)**	
		$C_{23}H_{46}NO_7P$	**LysoPE(18:1)**	
		$C_{23}H_{42}NO_{10}P$	**LysoPE(18:3/3-OH)**	
		$C_{25}H_{48}NO_7P$	**LysoPE(20:2)**	
		$C_{25}H_{48}NO_7P$	**LysoPE(20:2)**	
		$C_{25}H_{44}NO_7P$	**LysoPE(20:4)**	
		$C_{27}H_{44}NO_7P$	**LysoPE(22:6)**	
		$C_{27}H_{50}NO_7P$	**LysoPE(22:3)**	
		$C_{27}H_{48}NO_7P$	**LysoPE(22:4)**	
	 **Ex.: LysoPE(20:2)**	**$C_{27}H_{46}NO_7P$**	**LysoPE(22:5)**	
		$C_{27}H_{46}NO_7P$	**LysoPE(22:5)**	
		$C_{27}H_{44}NO_7P$	**LysoPE(22:6)**	
		$C_{28}H_{47}NO_9P$	**C20:2-diGly-PE**	
	Glycerophosphocholine	$C_{23}H_{42}NO_7P$	PC(15:3)	
	Sulfoethanolamine	**$C_{16}H_{35}NO_5S$**		
Entrance Destination	**Sulfoethanolamine**	**$C_{10}H_{19}NO_5S$**	**SE(8:1)**	
		$C_{11}H_{23}NO_5S$	**SE(9:0)**	
		$C_{12}H_{25}NO_5S$	**SE(10:0)**	
		$C_{13}H_{25}NO_5S$	**SE(11:1)**	
		$C_{14}H_{23}NO_4S$	**SE*(12:4)**	
	 **Ex.: SE(8:1)**	**$C_{19}H_{29}NO_7S$**		
		$C_{20}H_{41}NO_4S$		
	Purine	$C_{10}H_{13}N_5O_8S$	Guanosine, sulfate	
		$C_{12}H_{17}N_5O_8S$	Guanosine, N-dimethyl-, sulfate	
	Glycerophosphoethanolamine	**$C_{21}H_{44}NO_7P$**	**LysoPE(16:0)**	
	Oxilipins	$C_{18}H_{37}ClO_{10}$		

4. Discussion

Previous studies have observed local variations of fouling communities between different parts of harbors/marinas, which have been linked to pollution gradients (Rondeau et al. in press; Je et al. 2004; Ryu et al. 2011; Kenworthy et al. 2018b). The associated mechanistic process might be linked to a selective pressure exerted by such disturbances inducing locally distinct communities as well as local adaptation. To expose the causal link between local adaptation and environmental factors, reciprocal transplant experiments represent one of the best ways, though their use remains scarce in marine ecology due to technical difficulties to run such experiments (Kawecki and Ebert 2004; Angert and Schemske 2005; Chang and Marshall 2016; Sork 2018). Based on previous results in the very same marina few years before (Kenworthy et al. 2018b), we hypothesized a disturbance (here POPs and MTEs) gradient from the entrance to the inner of the marina and assumed that community structure would correlate with this gradient as previously observed. We also hypothesized that transplanted communities would be driven according to their new environmental conditions, thus revealing a causal link between the environment and community structure. We also hypothesized that such filter would be acting at different biological organization levels, potentially resulting in local adaptation which would be detectable in the community respiration, pollutant accumulation, and species metabolomes.

4.1 Contamination in sediments and animals

While no official common European guidelines for contamination in marine sediments do exist, we could compare our results with two North American and two European guidelines. The harbor sediments largely exceeded the various thresholds for some PAHs (chrysene, fluorene, fluoranthene and phenanthrene) as well as for total PCBs (33 congeners). This may constitute an important stress for local species, as the encountered concentrations would exceed a 50% probability of toxicity on model amphipods exposed to the sediment (US EPA 2005). MTEs pollution presents higher global values than recommended by CCME and US EPA guidelines, especially in the inner parts (Tab. 1).

552 According to the Sediment Quality Category (SQC) established from southern Spanish harbors (Guerra-
553 García et al. 2021), MTEs appear at good or moderate (Zn and Cu) levels. This is in agreement with the
554 French national monitoring network REPOM, for which the studied marina was sampled in 2011 and
555 2012 (and had levels below the lowest concentration category N1 for most contaminants; REPOM
556 2013). However, our present values and those recorded in 2016 by Kenworthy et al. (2018b), indicate
557 increasing Cu concentrations, exceeding the REPOM N1 (45 $mg.kg^{-1}$) category at various locations.

558 Among the different locations studied within the marina, contaminants did, as expected, vary between
559 the three locations spaced by less than 100 m. We observed significant differences in
560 benzo[*g,h,i*]perylene, fluorene and total PCBs concentrations among locations, with maximal values at
561 the inner location, but not organized as a gradient. MTEs in sediments, on the other hand, had maximal
562 concentrations in the innermost part of the marina and diminished towards the entrance. This trend
563 was particularly marked for Cu, Pb and Zn, two of which have been previously reported in 2016
564 (Kenworthy et al. 2018b). Such lower contaminant concentrations at the entrance might be linked to
565 the higher hydrodynamism and higher depth that we noted at this location (Sup. Fig. 1), as stronger
566 water mixing might dilute pollutants (Schiff et al. 2007).

567 Contaminant concentrations in the filter feeder *Bugula neritina* approximatively followed the same
568 trends as in sediments with, however, lower concentrations in general but high heterogeneity between
569 samples. An exception lays in pesticides which were equivalently concentrated or even higher (aldrin,
570 diazinon and chlordanes) in *B. neritina* samples than in the sediments. As previously reported for
571 various benthic species (Reynoldson 1987), the filter-feeding *B. neritina* might have bioaccumulated
572 these contaminants following sediment resuspensions due to meteorological or hydrodynamic events.
573 Interestingly, Cu presented low concentrations and no significant differences between locations in *B.*
574 *neritina* samples while it had a strong spatial variability and a higher pollution levels than most MTEs
575 in sediments (moderate, according to Guerra-García et al. 2021). Due to its high toxicity, it is a major
576 driver of harbor fouling communities and of the prevalence of non-indigenous species (Dafforn et al.

2008; Canning-Clode et al. 2011; Kenworthy et al. 2018b; Osborne and Poynton 2019). *Bugula neritina* is particularly resistant to Cu, even compared to other introduced bryozoans like *Watersipora* spp. commonly present in harbors (Piola and Johnston 2006a). Detoxification mechanisms may thus explain why despite high Cu concentrations in sediments, *B. neritina* had overall low concentrations.

4.2 Community analyses

Previous studies have shown that pollution gradients may structure communities by inducing local differences among them (Rondeau et al. in press; Je et al. 2004; Ryu et al. 2011; Kenworthy et al. 2018b). In the same marina as the present study, Kenworthy *et al*. (2018b) could correlate the high but varying levels of some sediments contaminants (Cu, Zn, and hydrocarbons) at small spatial scales (< 100 m) with heterogeneity in fouling communities of vertical pillars. While increasing the number of tested contaminants, the present study showed similar results with significant differences maintained throughout the whole duration of the experiment among communities from control panels (Fig. 2). Such similar spatial patterns maintained over time might be caused ecological selection (Vellend 2010). However, only an experimental approach such as reciprocal transplant may establish a causal relationship between the environmental conditions, community structure and biological functions (Osman and Whitlatch 2004; Cifuentes et al. 2010; Chang and Marshall 2016).

It was possible to observe a strong effect of the transplant on community structures. The global PERMANOVA revealed a strong effect of origin, destination, and their interaction (Treatment). The destination effect directly results from the transplant experiment as it reveals how location may select the presence and/or abundance of certain taxa, driving similarities between communities at the inner location. Here we confirmed differences in community composition between locations as previously observed (Je et al. 2004; Ryu et al. 2011; Kenworthy et al. 2018b), but we also demonstrated a causal relationship between location and community composition through the transplant experiment. Predation or recruitment dynamics may contribute to the observed differences among communities. However previous studies in the same marina have shown that despite spatially variable predation and

602 temporally variable recruitment dynamics, the impact of location remained constant (Rondeau et al.
603 in press; Gauff et al. 2022). Pollution and specifically Cu pollution has been thoroughly shown to highly
604 influence fouling communities (Piola and Johnston 2008; Canning-Clode et al. 2011; Kinsella and Crowe
605 2016). As pollution is highly variable among locations, this might indicate that the selective pressure
606 exerted by pollution in the inner part of the harbor may be major driver of community composition.

607 On the other side, population dynamics of *C. intestinalis* might explain the effect of panel origin. Before
608 transplant, *C. intestinalis* was completely covering panels from the entrance (Sup. Fig. 3 and Sup. Fig.
609 5), while at the end of the experiment, EC and E>I panels had a comparatively lower cover. Conversely
610 E>M panels were completely covered by very large individuals that were most likely the same
611 individuals noted before transplant (Sup. Fig. 5, middle). It is unlikely that this observation is linked to
612 the effect of predators on *C. intestinalis* as benthic predators have reduced access to the suspended
613 panels and as pelagic predators exert the strongest top-down effect at the middle of the marina (Gauff
614 et al. 2022), where *C. intestinalis* were abundant at the end of the experiment. Indeed, this species has
615 a rather short life cycle in the present harbor, recruiting twice a year (spring and late summer) and
616 living 2-4 months before reproducing and dying (Bouchemousse et al. 2016). The here observed
617 recruitment and die off of *C. intestinalis* is similar to previous observations in the same marina
618 (Rondeau et al. in press). Lethal and sublethal effects for this species were reported for some
619 environmental factors such as heavy metal pollution (Bellas et al. 2001, 2004). Considering the
620 contaminant levels recorded in the present harbor, the entrance may have been the most favorable
621 location for this species' high recruitment in the early phases of our experiment, then for completing
622 its reproduction cycle and subsequent mass mortality (hence its decline at the end of the experiment).
623 At the inner location, lethal effects of contaminants may explain the low presence of *C. intestinalis* in
624 inner controls and the sharp decline in E>I treatments. Individuals on E>M panels however, may have
625 encountered sublethal effects of pollution delaying their reproductive cycle and explaining the high
626 cover by large individuals at the end of the experiment. This illustrates the origin effect observed in
627 the PERMANOVA. Niche preemption as priority effect may highly influence the historical contingency

of a community (Fukami 2015). Previous transplant studies on fouling communities have already demonstrated the importance of primary colonization on the subsequent community composition in harbors (Cifuentes et al. 2010; Chang and Marshall 2016). Stochastic colonization may also have a high structuring role (Chase and Myers 2011). *Ciona intestinalis* is a highly competitive species with a tendency to monopolize substratum and reduce diversity (Blum et al. 2007). Considering all this, the monopolization of space by *C. intestinalis* at the entrance before transplant may explain why communities originating from this location strongly differentiate from all others even if this species dies off if transplanted to the inner location.

The indicator species analysis revealed that species may be associated to certain origins or to certain destinations, which reflects the coexistence of those effects as observed at the community level. The indicator species associated to specific destinations revealed the causal effect between environment and community structure. *Cryptosula pallasiana* and *Austrominius modestus* seem both to be species associated with the inner location, as they were indicator species of this location before transplant and are indicator species of the inner destination (Tab. 3). In general, the inner part of the marina seems to be more frequently correlated or associated with encrusting species and NIS (see Fig. 2 and Tab. 3). The indicator species associated to specific origins reveal that historical contingency plays an important role in the community structure and species present on panels. Interestingly, none of the indicator species for any location before transplant is an indicator species for the same origin after transplant (Tab. 3). Some of the indicator species associated to origin effects (ex. *Mytilus edulis* or *Asterocarpa humilis*) were not detected at all before transplant. Two very notable indicator species, *B. neritina* and *W. subatra* (both introduced bryozoans), were associated to the middle origin. These were also the panels where their percent cover was often the highest compared to panels from the same destination and the panels for which introduced species cover was the highest (Sup. Fig. 3). This might indicate that once these species gain a foothold on the substratum, they persist even if disturbance levels diminish, which may pose problems for their management in fluctuating environments.

4.3 Local adaptation is revealed at various organization levels and functions

A population is locally adapted if local selective pressures result in differentiated phenotypes among populations of the same species (Williams 1966; Kawecki and Ebert 2004; Blanquart et al. 2013). In its most common definition, local adaptation requires genotypic differences underlying these adaptive phenotypes, excluding adaptive plasticity (Kawecki and Ebert 2004). Yet, transgenerational plasticity plays an important role in adaptation (Mousseau and Fox 1998; Ghalambor et al. 2007) and in recent literature transgenerational adaptive plasticity through epigenetics is increasingly included in studies focusing on local adaptation and adaptation in general (Richards et al. 2010a; Platt et al. 2015; Moler et al. 2018; Sork 2018). DNA-methylation can often be observed in association with environmental gradients (Dubin et al. 2015; Platt et al. 2015; Clark et al. 2018) and may contribute to local adaptation if it affects adaptive traits (Richards et al. 2010b; Moler et al. 2018). As DNA-methylation might increase genetic mutation on the considered gene (Klironomos et al. 2013), locally adaptive phenotypes might even become genetically fixed (Ghalambor et al. 2007). Considering the spatial and temporal scales relevant for our study we included transgenerational plasticity our definition of local adaptation. Here, we investigated several biological functions to see if the 'local vs foreign' criterion (fitness differences among transplanted and destination control individuals) for local adaptation was respected (Kawecki and Ebert 2004). For this, we chose to focus on metabolic metrics to discuss the potential fitness of organisms.

Increased stress due to disturbance may engage energetically costly regulatory mechanisms like osmoregulation or detoxification (Roast et al. 1999; Pook et al. 2009; Lenz et al. 2011), which may in turn increase respiration (Kenworthy et al. 2018b). Here we did observe higher respiration rates in IC, M>I and E>I communities, which could indicate that the inner location may constitute a high stress environment for them. Interestingly, community respiration from the E>I treatment is even higher than the IC respiration, which would indicate an even higher stress level for these communities. This would be consistent with a 'foreign' disadvantage of these communities compared to IC (locals) and be a

potential indicator that at least some species might be locally adapted to the environmental conditions. Respiration was 3-5 times higher than what has been reported in the literature (Bakke and Skjoldal 1979; Lenz et al. 2011; Kenworthy et al. 2018a; Oh et al. 2020). Bacterial respiration might thus contribute to the elevated values at the inner location as it may account for between 30% and 60% of community respiration (Smith 1973; Martínez-García et al. 2013).

Contamination in animal tissues may be an important measure to be accounted for, as it may impact fitness through reproduction and survival (Piola and Johnston 2006a; b; Ruiz et al. 2011). In a recent meta-analysis encompassing over 300 species, pollution has been demonstrated to be the anthropic disturbance inducing the strongest phenotypic changes, potentially leading to rapid evolution (Sanderson et al. 2022). Here, the contaminant composition of *B. neritina* (see Sup. Fig. 2, Tab. 1, 2 and Sup. Tab 1) is mainly driven by MTE and PAH. Consequently, the EC (control at the least contaminated location) had a significantly lower normalized concentration than the two other controls (Fig. 1), but the transplant had a strong effect on *B. neritina* normalized contaminant concentrations: whatever their origin, individuals transplanted towards the entrance showed the lowest concentrations, while individuals transplanted to the inner location had systematically higher concentrations (Fig. 1). Interestingly, it is also possible to observe a differential accumulation of contaminants among individuals transplanted to the inner (most contaminated) location, with E>I individuals having a significantly higher concentration than IC and M>I individuals (Fig. 1). This might be an indication for local adaptation at very small scale (< 100 m) for this species, where individuals from less contaminated locations (entrance) may have a lower detoxification activity (or higher contaminant uptake) compared to their counterpart in contaminated locations (inner). There are many molecular and cellular mechanisms used for detoxification which can be explained by genetic adaptation and differences in expression regulation of detoxification pathways (Rainbow et al. 1990; Janssens et al. 2009). This species has been shown to have differential resistance to Cu among populations (Piola and Johnston 2006b). Maternal effects seem to have a particularly strong effect on *B. neritina* facing spatial variability of environmental factors (Marshall 2008; Burgess and Marshall 2011). Local adaptation

704 through epigenetics and maternal effects might explain the differential accumulation/detoxification of
705 this species in our case and need to be further explored.

706 Environmental conditions and pollution levels may be an important driver of the metabolome of a
707 species (García-Sevillano et al. 2015; Xue et al. 2019). The metabolomes of *B. neritina* and *C. intestinalis*
708 were significantly influenced by location and transplant. For *B. neritina,* it was possible to observe
709 differences in the metabolome among locations (IC distinct from other controls). Furthermore, a clear
710 pattern linked to the transplant could be observed (distinct metabolomes among panels exchanged
711 between the inner and entrance locations with their respective controls). For *C. intestinalis,* a clear
712 difference between metabolomes of EC and E>I individuals has been identified, but due to the absence
713 of IC metabolomes (not enough individuals for sampling), no clear patterns could be highlighted for
714 this species. Destination effects on the metabolome of *C. intestinalis* and *B. neritina* showed that both
715 species were able to modify their metabolism to accommodate for new environmental conditions.
716 Changes in the metabolome might be a direct response to variable contamination levels among
717 locations. For *B. neritina*, this destination effect however coincides with an origin effect since the E>I
718 metabolome was not only distinct from EC, but also from IC. This effect is even more visible for
719 metabolomes originating from the middle, which were different from MC and from both their
720 destination controls (EC and IC). These results are in accordance with the normalized pollutant
721 concentration in *B. neritina,* whose entrance individuals transplanted to the inner location seem to
722 present a lower detoxification activity (or higher pollutant uptake) compared to the inner control. Since
723 these activities are dependent on metabolic pathways (Rainbow et al. 1990; Janssens et al. 2009), they
724 should also manifest in the metabolome and at least partly influence it. Indeed, such a tendency may
725 be also reported from the expression profiles of some of *B. neritina*'s metabolites (Tab. 4). Glycero-
726 phospho-ethanolamines (GlyPE), more specifically Lyso-phospho-ethanolamines (Lyso-PE), have a very
727 distinct expression pattern with high expression in MC, M>E and EC. Expressions of this chemical family
728 varied according to their transplant destination (I>E > I>M > IC and EC > E>M > E>I). Interestingly, an
729 origin effect is also visible for Lyso-PE, since the concentration of E>I < IC < EC. This would further

730 indicate that local adaptation of this species may be present at this small spatial scale. Data from
731 literature suggest a potential link between GlyPE expression and reproductive activity. They have been
732 shown to vary with the reproductive cycle of the sponge *Oscarella tuberculata* (Schmidt, 1868), being
733 highly expressed during embryogenesis and early development (Ivanisevic et al. 2011). Moreover, lyso-
734 phospholipids also play a role in mammalian reproduction (Birgbauer and Chun 2006). Phospholipids
735 in general appear to be impacted by various types of pollutions in vertebrates and invertebrates (Al-
736 Salhi et al. 2012; García-Sevillano et al. 2015; Salihovic et al. 2015; Wang et al. 2017). Lyso-PE levels
737 have an almost exact opposite distribution than pollution levels in *B. neritina* indicating a negative
738 effect of pollution on Lyso-PE levels. It is possible to assume that the inner, more polluted location is
739 metabolically at higher cost for *B. neritina* due to higher energy cost of detoxification, leaving less
740 resources to allocate to reproduction.

741 Although it is difficult to make clear statements upon the metabolome of *C. intestinalis*, the analysis of
742 its metabolome allowed to identify several VIP as glycerophosphocholines (GlyPC) and GlyPE. They had
743 a similar distribution pattern to *B. neritina*'s ones. However, GlyPC and GlyPE levels in *C. intestinalis*
744 had a slightly lower expression at the EC compared to I>E, M>E and compared to MC (Sup. Tab. 3).
745 Their expression profile would be compatible with the low survival of *C. intestinalis* when transplanted
746 from the entrance to the inner location and the delay in reproductive activity due to less favorable
747 conditions when transplanted to the middle (Sup. Fig. 3, Sup. Fig. 5). This suggests that
748 glycerophospholipids may be excellent markers of environmental stress linked to pollution across
749 multiple animal classes, since they seem to be linked to a tradeoff between reproduction and
750 detoxification. Subsequent studies could use targeted metabolomics to assess levels of these
751 molecules in several study species.

752 Here we show strong indications for local adaptation, since the 'local vs foreign' criterion is satisfied
753 for the community respiration, contaminant accumulation and the metabolome of *B. neritina*. Our
754 results do however not satisfy the 'home vs away' (fitness differences among transplanted and origin

755 control individuals) criterion since I>E individuals had lower pollutant concentrations and higher Lyso-
756 PE levels than IC. This is due to differences in the intrinsic habitat quality, which is why the 'foreign vs
757 local' criterion is the foremost one to satisfy to conclude on local adaptation (Kawecki and Ebert 2004).
758 While the 'locals vs foreign' criterion may be an indicator for local adaptation, it is here, however, not
759 entirely possible to exclude within-generation plasticity (*i.e.*, acclimation) and common garden
760 experiments may be used to disentangle (Kawecki and Ebert 2004; Thorpe et al. 2005; De Villemereuil
761 et al. 2016; Moler et al. 2018). However, due to the short generation time of our studied communities
762 (months) and the duration of our experiment, and due to the strong effect of pollution on individual
763 fitness and survival, it is likely that acclimation after transplant should occur quickly. The remaining
764 'origin effect' may thus mostly be linked to more anchored phenomena. Strong selective forces are
765 primordial in generating local adaptation and contemporary evolution for introduced species (Kawecki
766 and Ebert 2004; Colautti and Lau 2015). Pollutants constitute a strong selective filter that can act on at
767 the community level (Je et al. 2004; Canning-Clode et al. 2011; Ryu et al. 2011), but also on the species
768 which might develop hereditary resistances (Piola and Johnston 2006b; Marshall 2008; McKenzie et al.
769 2011). While for the E>I treatment the 'foreign' disadvantage is striking, it is much more subtle for the
770 I>E treatment. However, if we use the detoxification activity as example, transplant from the inner to
771 the entrance should lead to a fitness disadvantage compared to EC 'locals'. Detoxification depends on
772 costly metabolic pathways since these require trade-offs between metabolism and other activities
773 (Handy et al. 1999; Pook et al. 2009). High detoxification activity in less polluted environments could
774 thus impair fitness and cause a competitive disadvantage (wasted energy) even if we were not able to
775 measure it. This may explain why glycerophospholipids, which we presume as fitness markers, are
776 lower in the I>E treatment compared to EC for *B. neritina*, which would satisfy the 'foreign vs local'
777 criterion in both directions. Many introduced species seem to be exclusive to anthropic structures and
778 do not 'escape' to natural habitats (Glasby 1999; Marins et al. 2010; Simkanin et al. 2012), which could
779 at least partially be explained by such disadvantage.

780

5. Conclusion

The present results highlight strong small-scale spatial variation of contaminants in sediments and animal samples. The community composition did not only vary in space in accordance to pollution as it has already previously been observed (Kenworthy et al. 2018b), but it was causally linked to environmental conditions as shown by the changes in community composition after transplant. However, this destination effect is concomitantly associated with an effect of origin location indicating that historical contingency and colonization succession may still play an equally crucial role. Our results provide strong evidence for local adaptation at a very small spatial scale (< 100 m) since community respiration, pollutant content of *B. neritina* and the metabolome of two species were impacted. We expose a clear disadvantage for the 'foreign' condition for individuals transplanted from the entrance to the inner, more polluted, location in community respiration, pollutant accumulation and metabolome. As the considered distances are lower than the potential dispersal distances of the studied species (Keough and Chernoff 1987; Svane and Havenhand 1993; Marshall and Keough 2003; Shanks et al. 2003; Collin et al. 2013), the local adaptation observed here may even constitute a case of microgeographic adaptation (Richardson et al. 2014). Dispersion and preferential settlement observed in fouling species (Mckenzie et al. 2012; McDonald et al. 2014) might also accentuate local differences if preferential settlement happens according to the individual phenotype (Bierne et al. 2003). While it might be possible that the studied species retained certain plastic traits from their original location, time after transplant was long enough for transplanted communities to acclimate to their new environment (one must consider that fouling communities are rather short-lived, mostly months). We consider that the observed differences are thus likely profoundly anchored in the local populations and may be regarded as local adaptation. The here observed results may thus indicate that the disturbance gradient in marinas might constitute a staple for selecting pollutant-resistant species and populations and cause local adaptation, which may explain why many NIS remain exclusive to anthropic structures and marinas. However, in natural and urbanized habitats surrounding marinas, pollution may occur in pulses due to wash off and water mixing events. Such pulse-pollution has

already been shown to have drastic impacts on settled communities (Johnston and Keough 2000, 2002; Johnston et al. 2002). This highlights the importance of conducting further studies into small scale local adaptation, using reciprocal transplant and common garden experiments to determine if the selective pressure in marinas might play an important role in restricting many introduced species to marinas and similarly polluted environments; or if it confers an advantage in areas with variable pollution levels.

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Supplementary material

Pollution gradient leads to local adaptation and small-scale spatial variability of communities and functions in an urban marine environment

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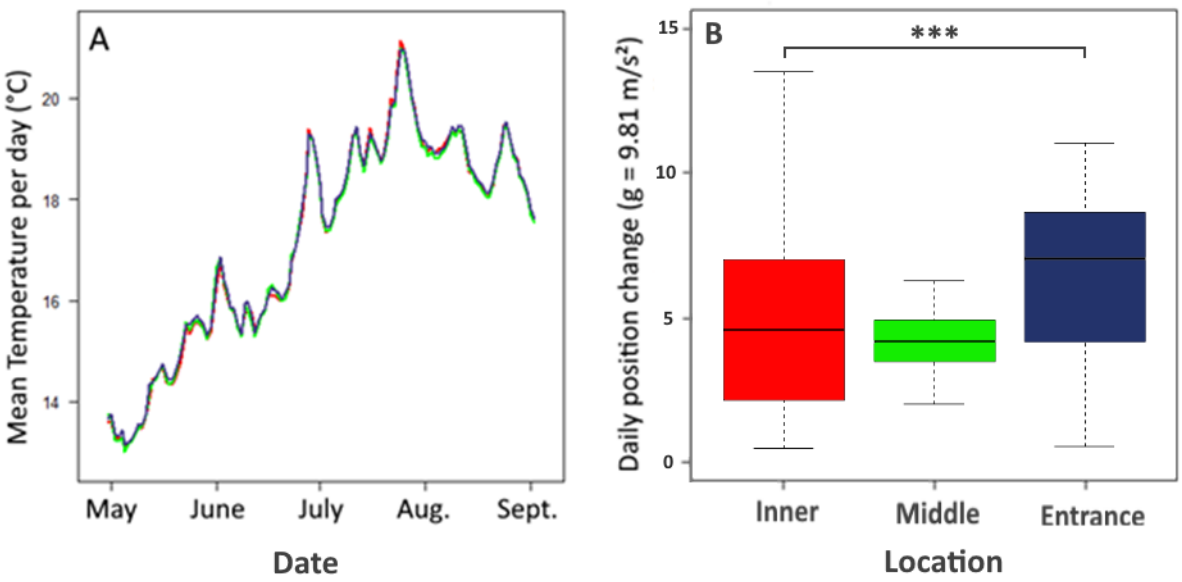
Abstract. Urbanization of coastal habitats, of which harbors and marinas are the paragon, has led to various ecological paradigms about their functioning. Harbor infrastructures offer new hard substrata that are colonized by a wide variety of organisms (biofouling) including many introduced species. These structures also modify hydrodynamism and contaminant dispersal, leading to strong disturbance gradients within them. Differences in sessile community structure have previously been correlated to these gradients at small spatial scale (< 100 m). Local adaptation might be involved to explain such results, but correlation is not causation. The present study aims to understand the causal link between the environmental gradients and community structure through a reciprocal transplant experiment among three sites of a marina (inner, middle, entrance). Our results highlighted strong small-scale spatial variations of contaminants (trace metals, PCB, pesticides, and PAH) in sediments and animal samples which has been causally linked to changes in community composition after transplant. But historical contingency and colonization succession also play an important role. Our results provided strong evidence for local adaptation since community structure, respiration, and pollutant uptake in *Bugula neritina,* as well as the metabolomes of *B. neritina* and *Ciona intestinalis* were impacted by the transplant with a disadvantage for individuals transplanted from the entrance to the inner location. The here observed results may thus indicate that the disturbance gradient in marinas might constitute a staple for selecting pollutant-resistant species and populations, causing local adaptation. This highlights the importance of conducting further studies into small scale local adaptation.

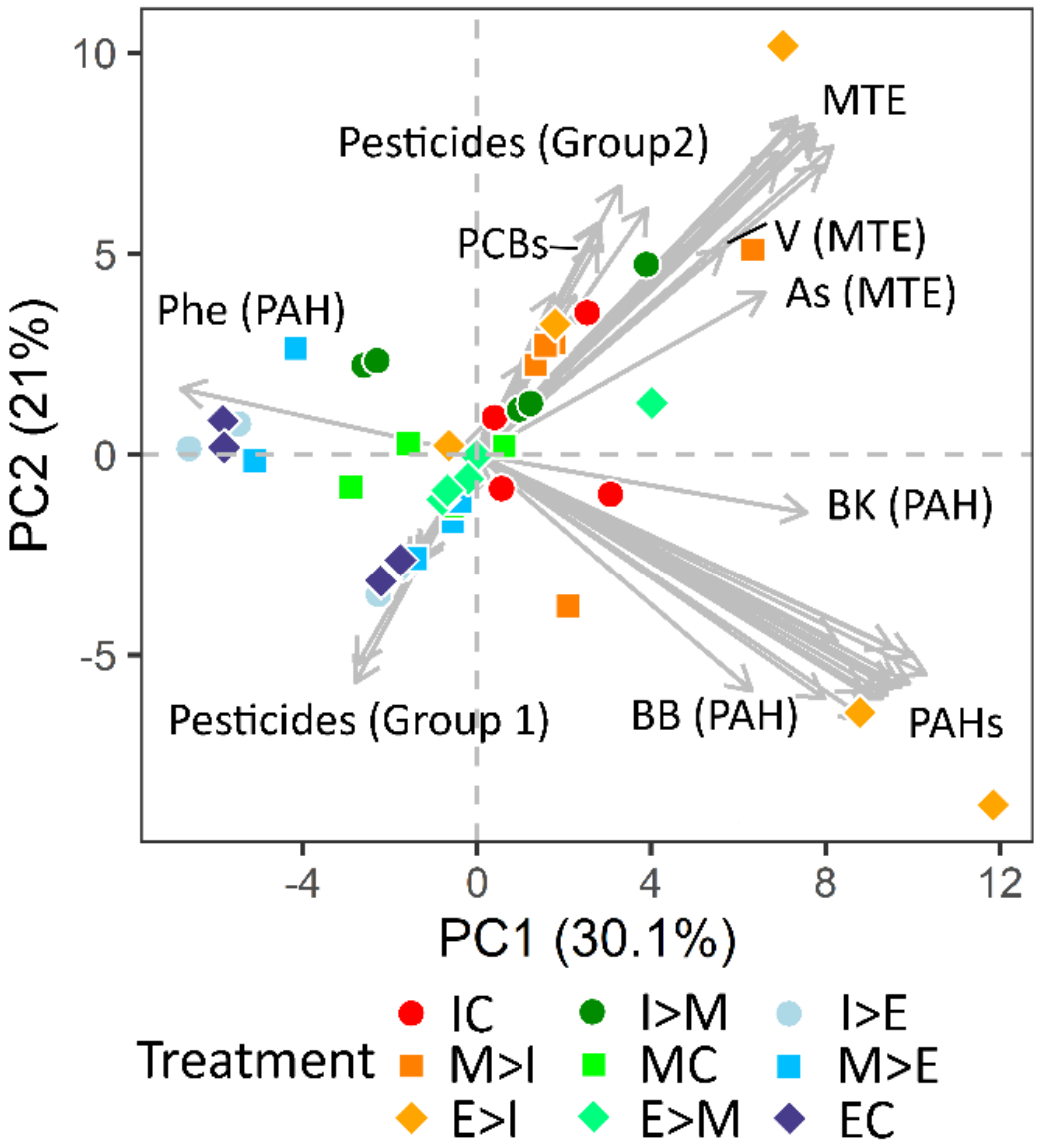
Keywords: local adaptation, fouling, pollution, metabolomics, respiration, marinas



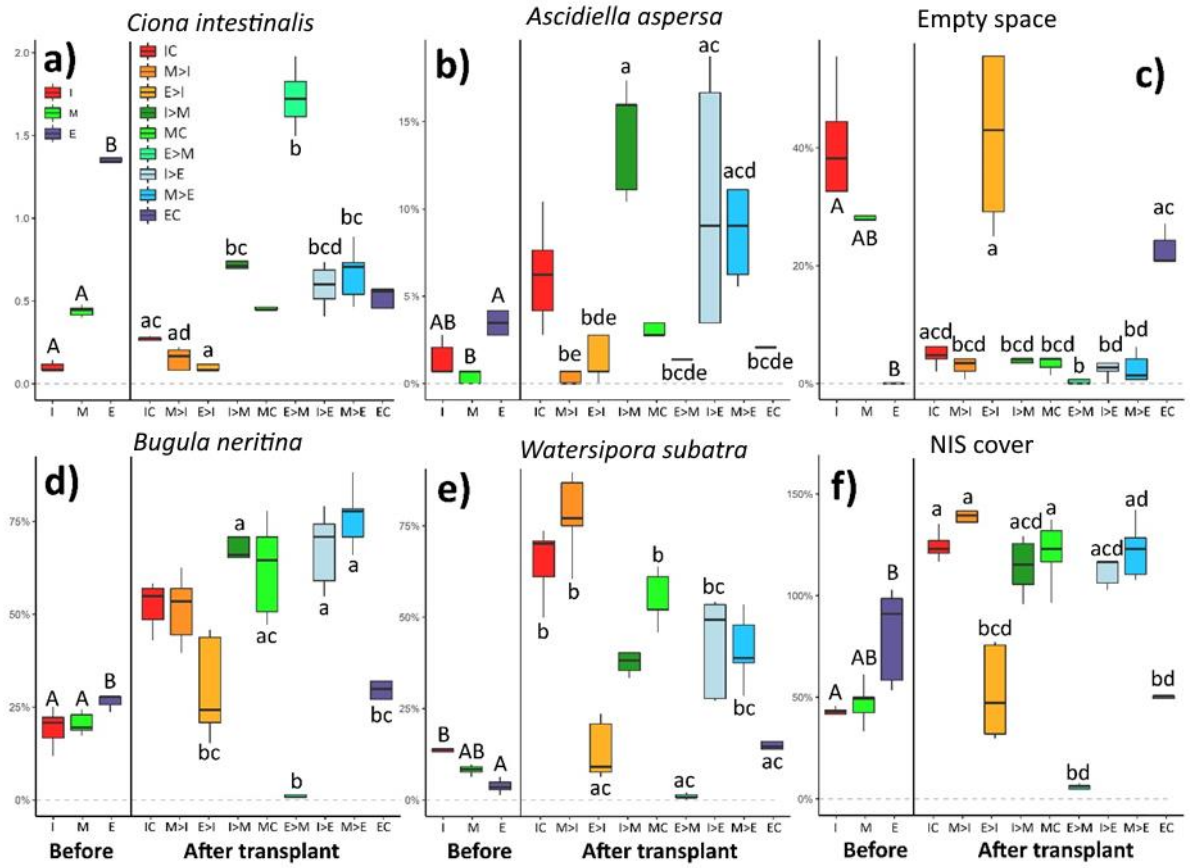
A. Fig. 1: Temperature curve (A) and boxplot of hydrodynamism (B) at the three locations. Red: Inner; Green: Middle; Blue: Entrance. For Temperature, the curves overlap although significant differences between pontoons were detected (LME, $t = 5.33$; $p < 0.05$). On a biological perspective, the differences were however negligible (< 0.1°C). Daily position change in g (9.81 $m.s^{-2}$) calculated from the difference between tridimensional vectors at T_n and T_{n-1}, revealed a significant higher hydrodynamism at the entrance compared to the inner location (LME; $t = 3.61$; $p < 0.001$).

A. Tab. 1: Mean values (µg.kg $^{-1}$ = ppb) with standard deviation of pesticide concentrations in the sediments of the three locations (n = 3) and in the bryozoan *Bugula neritina* control individuals (n = 5). Stars indicate significant differences between locations (Kruskal-Wallis test; *: $p < 0.05$). Sediment quality guidelines provided by the Canadian Council of Ministers of the Environment (CCME 1999; ISQG: Interim Sediment Quality Guideline; PEL: Probable Effect Level); and the United Stated Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples) are indicated. Bold font for reference values: threshold exceeded at least at one location.

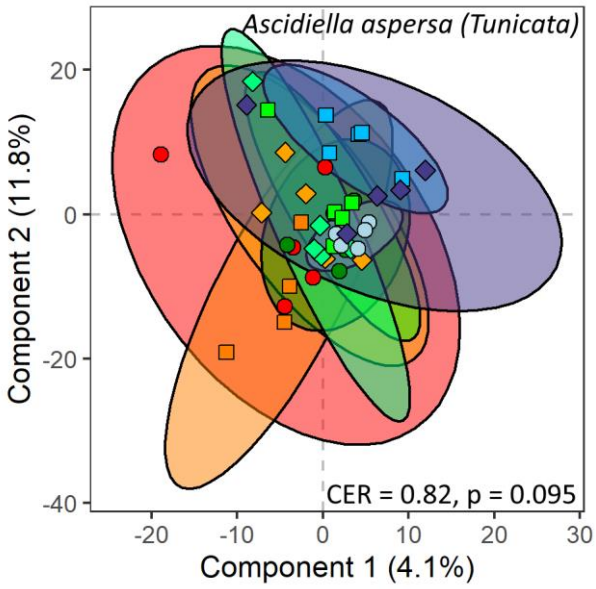
Pesticides (µg.kg^{-1})		**Location mean ± SD**			**K-W**	**CCME**		**US EPA**	
		Inner	**Middle**	**Entrance**	**test**	**ISQG**	**PEL**	**T20**	**T50**
Aldrin	Sediment	0.65 ± 0.2	0.81 ± 0.1	0.14 ± 0.1	ns				
	B. neritina	1.31 ± 0.1	0.89 ± 0.1	1.76 ± 0.3	*				
Cis_chlordane	Sediment	0.06 ± 0.01	0.06 ± 0.01	0.01 ± 0.03	ns				
	B. neritina	1.07 ± 0.3	2.03 ± 0.3	1.43 ± 0.3	ns				
Trans_chlordane	Sediment	1.2 ± 0.1	0.12 ± 0.03	0.03 ± 0.1	ns				
	B. neritina	6.15 ± 0.8	5.68 ± 0.7	7.56 ± 0.9	ns				
tChlordane	Sediment	1.26 ± 0.1	0.18 ± 0.04	0.04 ± 0.1	ns	2.26	4.79		
	B. neritina	7.22 ± 1.2	7.71 ± 0.9	8.99 ± 0.9	ns				
Diazinon	Sediment	0.57 ± 0.1	0.74 ± 0.3	0.28 ± 0.1	*				
	B. neritina	5.05 ± 0.3	3.76 ± 0.5	6.16 ± 0.2	*				
Dieldrin	Sediment	1.11 ± 0.1	1.25 ± 0.04	0.04 ± 0.3	*	**0.71**	4.3	**0.83**	2.9
	B. neritina	1.1 ± 0.2	4.31 ± 0.2	2.54 ± 0.7	*				
Endosulfan- 2	Sediment	14.1 ± 0.8	2.87 ± 0.5	13.2 ± 2.1	ns				
	B. neritina	6.43 ± 0.8	5.53 ± 0.8	2.02 ± 0.3	ns				
Endosulfan-1	Sediment	3.09 ± 0.1	1.22 ± 0.1	0.07 ± 0.8	*				
	B. neritina	0.17 ± 0.04	0.8 ± 0.6	0.51 ± 0	ns				
Heptachlor	Sediment	1.24 ± 0.2	0.74 ± 0.1	0.14 ± 0.3	ns	**0.6**	2.74		
	B. neritina	0.22 ± 0.04	2.48 ± 0.3	2.6 ± 0.3	ns				
Heptachlor_epoxide_a	Sediment	40.2 ± 4.7	21.4 ± 5.7	19 ± 1.7	ns				
	B. neritina	1.54 ± 0.1	0.31 ± 0.1	0.57 ± 0.1	*				
Heptachlor_epoxide_b	Sediment	3.48 ± 0.3	2.97 ± 0.3	0.27 ± 0.05	ns				
	B. neritina	3.28 ± 0.1	1.49 ± 0.1	1.22 ± 0.1	*				
Isodrin	Sediment	0.6 ± 0.2	0.23 ± 0.03	0.03 ± 0.1	ns				
	B. neritina	0.74 ± 0.1	1.59 ± 0.5	0.69 ± 0.1	ns				
Lindane	Sediment	2.03 ± 0.2	1.97 ± 0.3	0.32 ± 0.2	ns	**0.32**	**0.99**		
	B. neritina	1.86 ± 0.4	2.08 ± 0.2	1.52 ± 0.4	ns				
Methoxychlor	Sediment	7.43 ± 1.3	5.16 ± 0.2	0.21 ± 0.2	*				
	B. neritina	2.97 ± 0.3	1.04 ± 0.2	4.09 ± 0.2	*				
pp'-DDD	Sediment	10.7 ± 0.7	9.58 ± 1.1	1.09 ± 0.3	*	**1.22**	**7.81**	**2.2**	19
	B. neritina	2.47 ± 0.1	3.09 ± 0.7	4.68 ± 0.5	*				
pp'-DDE	Sediment	17.6 ± 0.5	22.1 ± 0.3	15.5 ± 1.7	*	**2.07**	374	**3.1**	100
	B. neritina	8.88 ± 2.2	2.25 ± 0.6	4.39 ± 0.8	*				
pp'-DDT	Sediment	0.62 ± 0.1	0.1 ± 0.03	0.03 ± 0.1	*	1.19	4.77	1.7	11
	B. neritina	0.29 ± 0.04	0.23 ± 0.01	0.18 ± 0.05	ns				



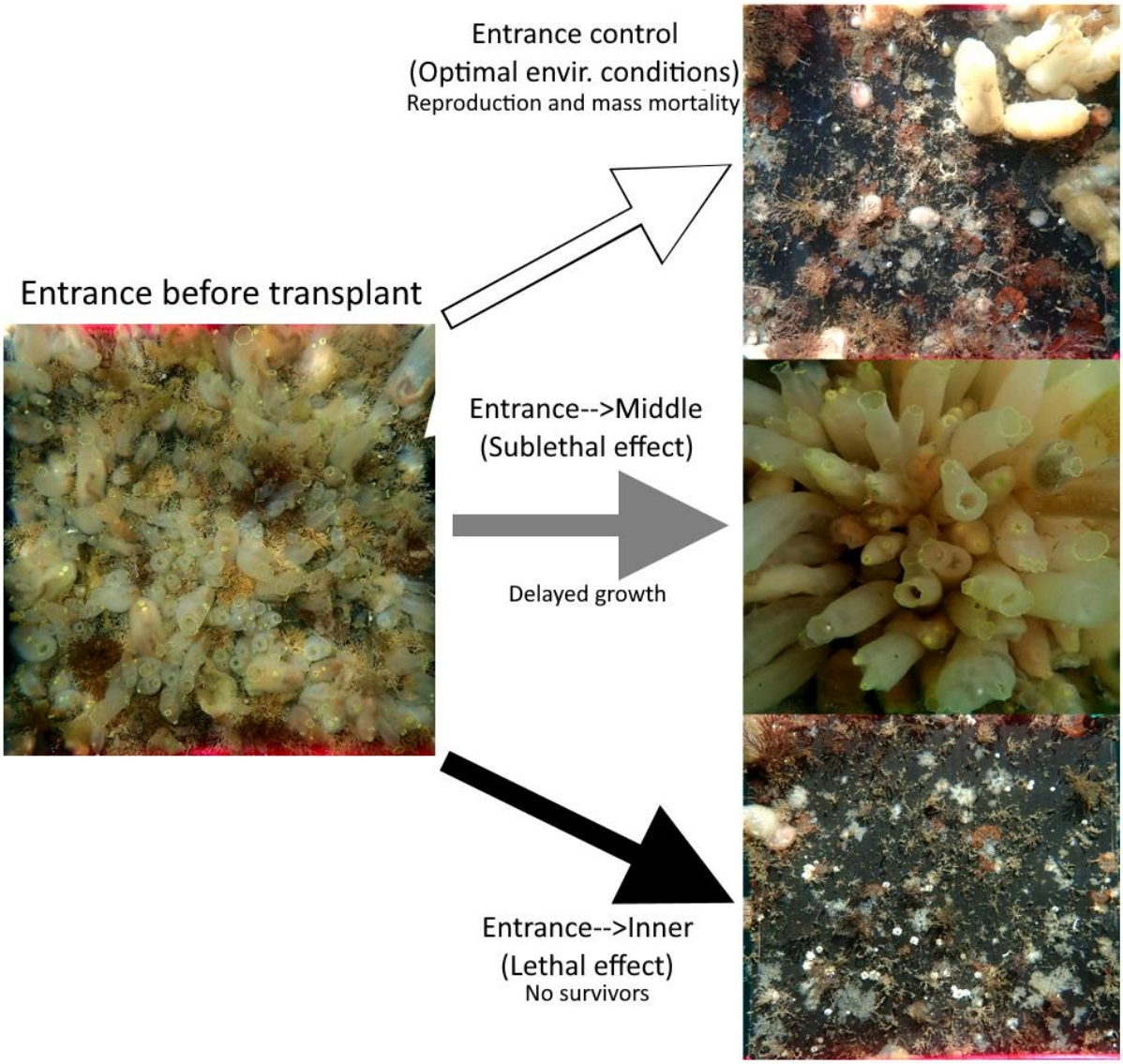
A. Fig. 2: Principal Component Analysis (PCA) biplot of contaminant concentrations in the bryozoan *Bugula neritina* according to their treatment. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated in the biplot according to their destination (red = inner; green = middle; blue = entrance) and origin (circle= inner, square = middle, diamond = entrance). MTE: all MTEs except As and V; PAH: all PAHs except Phe (Phenantrene), BK (Benzo[*k*]fluoranthene) and BB (Benzo[*b*]fluoranthene); Pesticides (Group 1): heptachlor, methoxychlor, pp.DDT, Endosulfan 1, Cis_chlordan, Trans_Chlordan; Pesticides (Group 2): heptachlor_epoxide_b, pp.DDE, Diazinon, heptachlor_epoxide_a, lindane, dieldrin.



A. Fig. 3: Boxplot of the cover of several important species before transplant (June) and 2 months after transplant (September). a) percent-cover of *Ciona intestinalis* (squareroot-transformed data) b) percent cover of *Ascidiella aspersa* c) percent cover of empty space without organisms, d) percent cover of *Bugula neritina* e) percent cover of *Watersipora subatra* and f) percent cover of introduced species. Cover can exceed 100% if species overlap (See methods). Treatments are labelled according to their origin followed (>) by their destination upon transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination). Statistic groups (Dunn Test) indicated by letter groups. Treatments before transplant were compared among them (Capital letters). Treatments 2 months after transplant were compared among them (lower case letters). Boxes without letters are NS with all other.



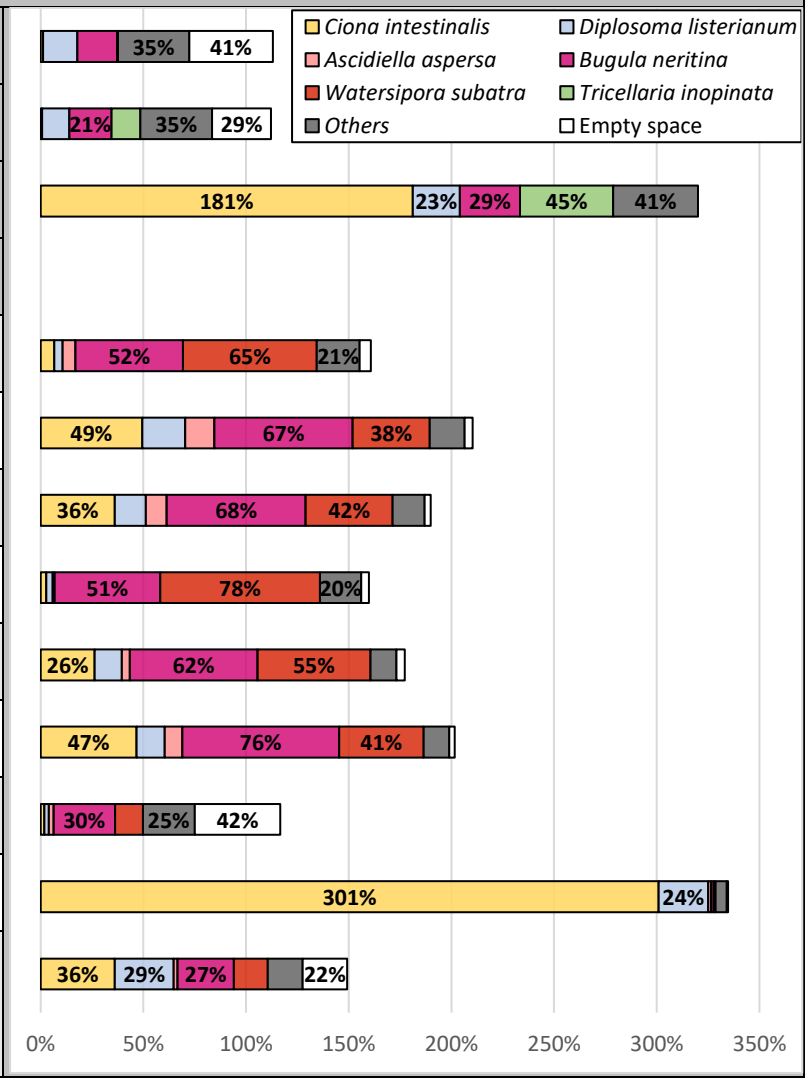
A. Fig. 4: Partial Least Squares Discriminant Analysis (PLS-DA) of the metabolome of *Ascidiella aspersa*. Ellipses indicate confidence intervals of 0.95 of each tratment (MVA test; p = 0.095, CER (Classification Error Rate) = 0.82). Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated in the biplot according to their destination (red = inner; green = middle; blue = entrance) and origin (circle= inner, square = middle, diamond = entrance).



A. Fig. 5: Photos of the panels from the entrance before transplant (single left) and after transplant: control (top right), to the middle (middle right) and to the inner location (bottom right). Hypotheses concerning the cover of *Ciona intestinalis* are indicated.

A. Tab. 2: Mean diversity indices (Species richness: S; Shannon index: H'; Pielou's evenness: J') and percent cover of the most abundant species for each treatment before transplant and 2 months after transplant. Treatments are labelled according to their origin followed (>) by their destination upon transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination). Cover can exceed 100% if species overlap (See methods).

Treat.	Mean diversity			% Cover of most abundant species
	S	H'	J'	
Before transplant				
IC	15.2	2.9	0.74	
MC	20	3.3	0.75	
EC	14.8	2.1	0.55	
After transplant				
IC	18.2	2.4	0.56	
M>I	14.6	2.0	0.52	
E>I	17.4	2.9	0.70	
I>M	18.4	2.6	0.63	
MC	16.4	2.3	0.58	
E>M	11	0.6	0.19	
I>E	17.6	2.5	0.61	
M>E	16.4	2.4	0.59	
EC	17.8	2.6	0.63	



A. Tab. 3: Variable Importance in Projection (VIP) scores associated to the PLS-DA of *Ciona intestinalis*. Molecules within a family are sorted with increasing number of carbons, number of oxygens, and number of unsaturations. Treatments are labelled in the heatmap according to their origin (top) followed by their destination (below) 2 months after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination). Heatmap legend on top of heatmap.

Chemical family	Molecular formula	Putative Molecular structure	Heatmap
			Low  High
			I C / M I / E I / I M / M C / E M / I E / M E / E C
Glycerophosphocholine derivatives	$C_{26}H_{44}NO_8P$	LysoPC(19:4-O)	NA
	$C_{29}H_{52}NO_9P$	LysoPC(22:2-O_2)	
	$C_{31}H_{52}NO_9P$	LysoPC(24:4-O_2)	
Glycerophosphocholines	$C_{28}H_{48}NO_7P$	LysoPC(20:5)	
	$C_{30}H_{50}NO_7P$	LysoPC(22:6)	
Glycerophospho-ethanolamines	$C_{23}H_{46}NO_7P$	LysoPE(18:1)	
	$C_{23}H_{44}NO_7P$	LysoPE(18:2)	
	$C_{25}H_{44}NO_7P$	LysoPE(20:4)	
	$C_{25}H_{42}NO_7P$	LysoPE(20:5)	
	$C_{27}H_{44}NO_7P$	LysoPE(22:6)	

Metabolome extraction and mass spectrometry methods

Three species occurring at all three locations in the harbor were sampled for metabolomic analyses on every settlement plate: two sufficiently large colonies of the bryozoan *Bugula neritina* and one individual of the two solitary ascidians *Ciona intestinalis* and *Ascidiella aspersa* were sampled. They were immediately frozen in dry ice in the marina, and then stored at -80°C at the laboratory. The samples were then all freeze-dried and mechanically ground to dust. The metabolome of the three species was extracted out of 40 mg of dry powder with 3 times 1 mL MeOH/DCM (CH_2Cl_2; 1:1) and ultrasonication during 2 min at room temperature. The supernatants were filtered with PTFE syringe filters (13 mm, 0.22 µm, Restek®) and transferred to vials containing silica powder (40 mg, C-18 Polygoprep® 60-50, Macherey-Nagel®). The resulting volume of 3 mL of extract was dried, first under the hood (for DCM evaporation) and secondly under vacuum (SpeedVac®, SPD111V, Thermo Scientific® Savant). The dried extract fixed on silica was fractioned by SPE on C-18 cartridges (Strata® C18-E 55 µm, 70 Å, 500 mg / 6 mL, Phenomenex®). The cartridges were flushed two times with 2 x 5 mL MeOH and 2 x 5 mL DCM, intensively dried under vacuum and conditioned with 2 x 5 mL H_2O. The extracts were transferred onto the cartridges, rinsed with 3 x 5 mL H_2O to remove salts and eluted with 5 mL MeOH and 5 mL DCM. The MeOH fractions were dried under vacuum, while the DCM fractions were dried under the hood, and stored in a freezer. Samples were resuspended in 500 µL MeOH for the polar fractions and in 500 µL DCM for non-polar fractions before analysis in LCMS and GCMS, respectively. For each species and fractions, a pool sample was created by mixing 30 µL of each sample. Three blank samples, that underwent the entirety of the extraction and fractioning process, were made for each species and fraction.

DCM fractions were analyzed with a gas chromatograph (7890B GC System - 7693 autosampler, Agilent Technologies®) coupled to a mass selective detector (5977A MSD, Agilent Technologies®). A volume of 1 µL was injected in splitless mode at 250 °C. Metabolites were separated on a HP-5MS 5% Phenyl-Methyl Siloxane column (30 m × 0.25 mm, 0.25 µm, Agilent Technologies®) using helium as carrier gas. The temperature was fixed at 40°C for 5 min and progressively raised from 40 °C to 300 °C (10 °C.min^{-1}). The fixed flow rate was set to 1 mL.min^{-1} all along the run. A solution of C8-C20 and C21-C40 alkanes (Fluka Analytical) was injected for the determination of compound retention indexes.

GC-MS Agilent data was exported into CDF format using MSD Chemstation (F.01.001903, Agilent Technologies®) and processed in R using the 'eRah' package (version 1.1.0; Domingo-Almenara et al. 2016) following these different steps : preprocessing, peak deconvolution [min. peak width = 5, min. peak height = 500, noise threshold = 100, avoid processing m/z = c (73:75,147:149,207:208)], peak alignment (min. spectra correlation = 0.90, max. time distance = 20, *m/z* range = 40:500) and missing

compound recovery (compound detected in > 2 samples). CGMS compound annotation was performed by comparison of mass spectra with those of the NIST 2014 database and Kovàts' index (van Den Dool and Dec. Kratz, 1963).

The injections in LCMS were performed with an UHPLC instrument (Dionex Ultimate 3000 equipped with RS Pump, autosampler, thermostated column and UV diode array, Thermo Scientific®) coupled to a Quadrupole Time of Flight spectrometer (QqToF) equipped with an ESI source (Impact II, Bruker Daltonics®). UHPLC separation was conducted on an Acclaim™ RSLC 120 C18 column (150 mm x 2.1 mm, 2.2 µm, ThermoScientific®) at 40 °C with an elution rate of 0.5 $mL.min^{-1}$. We used water (LC/MS grade, Carlo Erba®) with 0.1% formic acid (A) and acetonitrile (LC/MS grade, Carlo Erba®) with 0.1% formic acid (B) as chromatographic solvents. The chromatography cycled through 1) a mix of 5:95 of A:B during 2 min, 2) a linear increase up to 100% B during 8 min followed by 100% B during 4 min, 3) then a return to initial conditions (5:95, A:B) for 3 min for a total runtime of 17 min per sample. An injection of 2 µL in $LCMS^{1+}$ and $LCMS^{2+}$ (positive mode) and of 0.2 µL in $LCMS^{1-}$ and $LCMS^{2-}$ (negative mode) was performed for *B. neritina.* For the other species, the injection volume was of 1 µL in $LCMS^{1+}$ and $LCMS^{2+}$ and of 0.2 µL in $LCMS^{1-}$ and $LCMS^{2-}$. LCMS parameters were set as follows: nebulizer gas, N_2 at 3.5 bars; dry gas, N_2 at 12 $L.min^{-1}$, capillary temperature at 200°C and voltage at 3500 V. A full scan from 50 to 1200 mass units at 2 Hz was performed for both modes. The spectrometer was calibrated with a formate:acetate solution in basic media (Bruker) and before each sample for mass calibration. The Pooled sample of each species was injected at regular intervals (every 9 samples) to correct for possible mass spectrometer drift during the sequence. Blank samples were injected to filter for ions linked to the solvent and the extraction process.

LCMS profiles were automatically recalibrated using internal calibration in 'Bruker Compass DataAnalysis' (version 4.3) assuring a *m/z* precision of 2-5 ppm on the mass range. The generated netCDF data were processed with the R package 'XCMS' (version 3.12; Smith et al. 2006). The first step consisted in peak picking for the detection of different features, followed by a retention time correction (obiwarp method), a grouping of features in a fixed time frame (5 s) and peak filling to integrate portions where peaks were initially absent. The report and data matrix were then generated in Microsoft Excel format. Ions were normalized in Microsoft Excel following an established protocol (Van Der Kloet et al., 2009). The normalized matrix was subjected to three consecutive filtering in-house steps on R. These filtering methods consist in filtering ions originating from the blanks based on signal/noise ratio (S/N = 10), filtering ions for which intensities were highly variable in the pooled sample (> 0.2), filtering ions that were auto-correlated (> 0.8) to discard redundancy.

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