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► To cite this version:

Robin P.M. Gauff, Dominique Davoult, Stephane Greff, Olivier Bohner, Jérôme Coudret, et al.. Pollution gradient leads to local adaptation and small-scale spatial variability of communities and functions in an urban marine environment. *Science of the Total Environment*, 2022, 838 (1), pp.155911. 10.1016/j.scitotenv.2022.155911 . hal-03670481

HAL Id: hal-03670481

<https://hal.sorbonne-universite.fr/hal-03670481v1>

Submitted on 17 May 2022

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

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

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

21

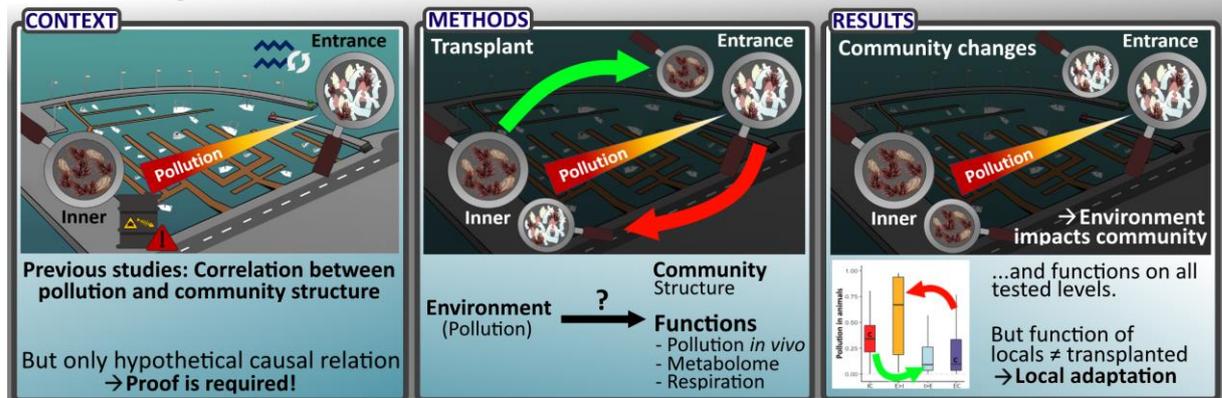
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25 Graphical abstract.

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



26

27 1. Introduction

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

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

57 Harbors are a major area of species introduction as they are at the end points of main introduction
58 vectors through maritime traffic (Mineur et al. 2008; Sylvester et al. 2011; Ferrario et al. 2017).
59 Introduced species, if they become invasive, sometimes completely restructure ecosystems, often
60 leading to the loss of biodiversity and ecosystem services (Pejchar and Mooney 2009; Johnston et al.
61 2015b; Walsh et al. 2016). Together with the cost of mitigating invasive species, this loss of services
62 can cause severe economic impacts, ranging from hundreds of millions to tens of billions dollars,
63 depending on the country (Lovell et al. 2006; Olson 2006; Jardine and Sanchirico 2018). Introduced
64 species seem to be favored in disturbance regimes (Hobbs and Huenneke 1992; Altman and Whitlatch
65 2007; Bulleri et al. 2016) as they are often described as opportunistic and more resistant to high and
66 drastic fluctuations. They have for instance been shown to be more resistant to extreme temperature
67 events (Zerebecki and Sorte 2011; Kelley 2014; Marie et al. 2017; Kenworthy et al. 2018a) which are
68 more common in harbors due to the reduced water volume and exchange with the open sea (Menniti
69 et al. 2020). Copper, used as active component of many antifouling coatings of ships, demonstrated its

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

75 Previous studies have observed local variation of fouling (native and introduced) communities
76 between different parts of harbors/marinas, which has been linked to pollution gradients (Je et al.
77 2004; Ryu et al. 2011; Kenworthy et al. 2018b). This may indicate that the selective pressure exerted
78 by disturbances in marinas may operate at small spatial scales, structuring the community and
79 potentially causing local adaptation at the species level. Such studies remain however putative
80 regarding the causal link between disturbance and community composition as they show correlative
81 results. As correlations between phenotypes and environmental conditions may be caused by
82 phenotypic plasticity, manipulative experiments are required to expose the link between concurrent
83 natural selection and observed variability (Kawecki and Ebert 2004; Thorpe et al. 2005). To expose the
84 causal link between local adaptation and specific environmental factors, reciprocal transplant
85 experiments should be conducted with one species or with entire communities (Kawecki and Ebert
86 2004; Angert and Schemske 2005; Chang and Marshall 2016; Sork 2018). It is then possible to reveal
87 the presence of local adaptation, if fitness differences exist between 'locals' originating from a studied
88 location and 'foreign' which were transplanted to this location ('locals vs foreign' criterion; Kawecki
89 and Ebert 2004; Martin et al. 2021). Additionally, the 'home vs away' criterion can be satisfied if
90 individuals at 'home' have higher fitness than their transplanted counterparts ('away'). This last
91 criterion is however not a strict requirement to conclude on local adaptation (Kawecki and Ebert 2004).
92 To occur, local adaptation requires a strong selective filter (Kawecki and Ebert 2004), such as
93 anthropogenic disturbances. If selective pressure is strong enough to overcome the homogenizing
94 effect of dispersion, local adaptation at smaller scales than the dispersal neighborhood of the
95 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

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

127

128 **2. Materials and methods**

129 2.1 Study site

130 The Marina du Château (48°22'43.4"N; 4°29'22.1"W) in Brest, France was chosen as study site. This
131 recreational marina, nested within the highly anthropized urban area of Brest, is integrated in a 250 ha
132 harbor including a commercial ($> 2 \cdot 10^6$ t.yr⁻¹ merchants, fishing and petroleum activities) and a military
133 (2nd biggest French arsenal) part, which occupies the major part of the city coastline. The marina is
134 characterized by numerous artificial substrata, colonized by fouling communities including several
135 introduced species (Kenworthy et al. 2018b). We focused this study on floating pontoons as they are
136 one of the most frequent substrata in the marina. In accordance with this previous study, the same
137 three locations (inner, middle and entrance; spaced < 100 m) were studied.

138

139 2.2 Reciprocal transplant experiment

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

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

158

159 2.3 Environmental assessment

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

$$166 \sum_{tn}^{t0} ([\vec{a}_x(t) - \vec{a}_x(t-1)] + [\vec{a}_y(t) - \vec{a}_y(t-1)] + [\vec{a}_z(t) - \vec{a}_z(t-1)])$$

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

170 (REML) were used (explaining temperature or hydrodynamism by location and using date as random
171 factor) with t-test with Satterthwaite's method from the 'lme4' and 'lmerTest' package in R (version
172 3.1-3, Zeileis and Hothorn 2002; version 1.1-26; Bates et al. 2015).

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

181 Sediment and animal MTEs samples were dissolved in a mixture of three-acids (HCl, HNO₃, HF; all
182 Suprapur grade). The solutions were then analyzed for Metallic Trace Elements (MTE) using a Sector
183 Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS; ELEMENT XR, ThermoElement) as in
184 Jacquet et al. (2021). The instrument was calibrated using standard solutions, and we used an external
185 calibration method with Indium as an internal standard. The efficiency of the dissolution procedure
186 and the quality of the SF-ICP-MS measurements were controlled by the determination of element
187 concentrations on Certified Reference Materials (MESS-4, SLRS-5, SLEW-3 and CASS-5). Concentrations
188 and the list of targeted MTEs is reported in Tab. 1.

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

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

200 For consistency, all contaminants were tested for differences among locations using a Kruskal-Wallis
201 test in R (version 4.0.3; R Core Team 2020) followed by a Dunn test from the 'FSA' package (version
202 0.8.32; Ogle et al. 2021), as conditions for the application of parametric tests were not always satisfied.
203 Reference values by the Canadian sediment quality guidelines (CCME 1999), by the US Environmental
204 Protection Agency (US EPA 2005), and by the French national monitoring network of water and
205 sediment qualities in maritime harbors (REPOM 2013) were added. For MTEs, an additional sediment
206 quality category (SQC) by Guerra-García et al. (2021) was reported. In order to integrate all
207 contaminants into one metric, normalized contaminant concentrations in *B. neritina* were used:

$$208 \quad C_{i(norm)} = \frac{(C_i - C_{min})}{(C_{max} - C_{min})}$$

209 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
210 values of contaminant concentrations. The normalized data was plotted in a boxplot for each
211 transplant and control treatment with 'ggplot2' in R (version 3.3.3; Wickham 2016). A two-level ANOVA
212 was used to test for the effect of Origin and Destination on the normalized concentration of all
213 contaminants in *B. neritina*, as well as for the effect of their interaction. A Tukey's honest significance
214 test (Tukey HSD; Tukey 1949) was conducted to test for differences of the normalized concentration
215 between treatments. To visualize the importance of individual contaminants in *B. neritina* samples, we
216 additionally conducted a Principal Component Analysis (PCA; 45 variables) with auto-scaled variables
217 using the R package 'FactoMineR' (version 1.34; Lê et al. 2008). PCAs are a highly powerful technique
218 for analyzing multinormal quantitative 'environmental' data (here contaminants in tissue samples;
219 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 ($> 0.4 \text{ points.cm}^{-2}$) 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

270 concentration was measured right after sealing off the chamber and at the end of incubation (between
271 14 and 23 min). The measured ΔO_2 (in $mg\ O_2 \cdot L^{-1}$) was then rated according to the volume of the
272 chamber, the incubation time, and the ash free dry mass (AFDM) to obtain the respiration in $mg\ O_2 \cdot h^{-1} \cdot g_{AFDM}^{-1}$. The AFDM was determined after the community analyses in the laboratory were finished. The
273 whole community was dried for one week at $60^\circ C$ for each panel. After a first mass measure, it was
274 burned at $520^\circ C$ for 6 h to allow to calculate the AFDM. For respiration, two aberrant values were
275 excluded from analysis. Respiration measurements were compared between treatments *via* an ANOVA
276 followed by a Tukey HSD test.

278

279 2.6 Metabolome assessment

280 Three species occurring at the three locations in the marina were sampled for metabolomic analyses:
281 two large colonies of the bryozoan *Bugula neritina* per panel and one individual per panel of the two
282 solitary ascidians *Ciona intestinalis* (Linnaeus, 1767) and *Ascidella aspersa* (Müller, 1776). For *B.*
283 *neritina* and *C. intestinalis*, only four samples could be constituted for the E>I. Additionally, the IC and
284 M>I treatment are missing entirely for *C. intestinalis* due to the absence of individuals for these
285 conditions. After sampling on the field, samples were immediately frozen in dry ice and then stored at
286 $-80^\circ C$. Samples were analyzed according to different mass spectrometry approaches. The detailed
287 extraction, injection protocols and Mass Spectrometry (MS) modalities (non-fragmenting modes
288 LCMS¹⁺, LCMS¹⁻; Fragmenting modes LCMS²⁺, LCMS²⁻; and GCMS) can be consulted in the
289 supplementary material. For each species, all features from the LCMS¹⁺, the LCMS¹⁻ as well as the GCMS
290 were combined into one matrix and treated with 'Metaboanalyst' (version 5.0; Xia et al. 2009). The
291 imported matrix (with less than 5000 features) was log transformed and auto scaled. We conducted a
292 Partial Least Squares Discriminant Analysis (PLS-DA) with class order assuming a gradient from the
293 inner to the entrance location, providing the best discrimination among treatments (Xia et al. 2009;
294 Kuhlisch and Pohnert 2015). In parallel a PPLS-DA driven permutation Model Validation Analysis (MVA)

295 as well as a pairwise MVA test using the 'RVAideMemoire' package in R (version 0.9-79; Hervé 2021)
296 were performed to test for statistical differences between treatments (10^4 permutations, 6
297 components). After revealing significant differences between treatments for *B. neritina*, the 100 first
298 Variable Important in Projection (VIP) scores were selected and tentatively annotated with the help of
299 the LCMS² (fragmentation) data. The annotated VIP were gathered in a table including an expression
300 heatmap ranging from the highest to the lowest ion intensity in LCMS¹ for each treatment. Mean
301 intensity was used when a feature was detected as a VIP in LCMS¹⁺ and LCMS¹⁻. Annotated metabolites
302 were sorted following their heatmap similarity in the dendrogram, while allowing compromise for
303 keeping chemical families together for clarity. Molecules within a family were sorted by numbers of
304 carbons, oxygens, and unsaturations. For *C. intestinalis* a more streamlined approach was used for
305 simplification as some treatments were missing. Using a molecular network generated on Global
306 Natural Products Social Molecular Networking (GNPS; version 1.3.15; Wang et al. 2016) and visualized
307 on 'cytoscape' (version 3.7.2), we localized clusters in LCMS²⁺ and LCMS²⁻ corresponding to
308 glycerophospholipids and only highlighted VIP within these clusters.

309

310 3. Results

311 3.1 Environmental characterization

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

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

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

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

335 **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
 336 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 <
 337 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;
 338 PEL: Probable Effect Level); the United States Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of
 339 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
 340 contamination with probable negative impact); and Sediment Quality category (SQC) by Guerra-García et al. (2021), are indicated. Bold font for reference values: threshold
 341 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							345
	<i>B. neritina</i>	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
	<i>B. neritina</i>	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
	<i>B. neritina</i>	0.2 ± 0.1	0.18 ± 0.1	0.07 ± 0.03	*							345
⁵² Cr	Sediment	57.4 ± 7.3	50.7 ± 11	43.6 ± 25	ns	52.3	160	49	140	90	180	Good
	<i>B. neritina</i>	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
	<i>B. neritina</i>	3.08 ± 3.5	1.1 ± 1.2	4.61 ± 5.7	ns							
⁵⁶ Fe	Sediment	13569 ± 1427	12639 ± 2980	10643 ± 6037	ns							
	<i>B. neritina</i>	521 ± 105	408 ± 138	172 ± 56	*							
⁵⁵ Mn	Sediment	150 ± 22	154 ± 29	134 ± 74	ns							
	<i>B. neritina</i>	22.6 ± 5.1	20.2 ± 10	8.1 ± 3.3	*							348
⁶⁰ Ni	Sediment	18.1 ± 1.8	16.6 ± 3.8	14.3 ± 8.2	ns			15	47	37	74	Good
	<i>B. neritina</i>	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
	<i>B. neritina</i>	5.3 ± 2.3	2.71 ± 0.5	1.56 ± 0.4	**							
⁴⁷ Ti	Sediment	2283 ± 291	2285 ± 506	1923 ± 1094	ns							
	<i>B. neritina</i>	6.15 ± 4	3.44 ± 1.5	1.53 ± 0.7	*							
⁵¹ V	Sediment	89.8 ± 11	75.6 ± 19	64.6 ± 37	ns							
	<i>B. neritina</i>	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
	<i>B. neritina</i>	58.5 ± 16	53.5 ± 13	31.9 ± 4.2	*							

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

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

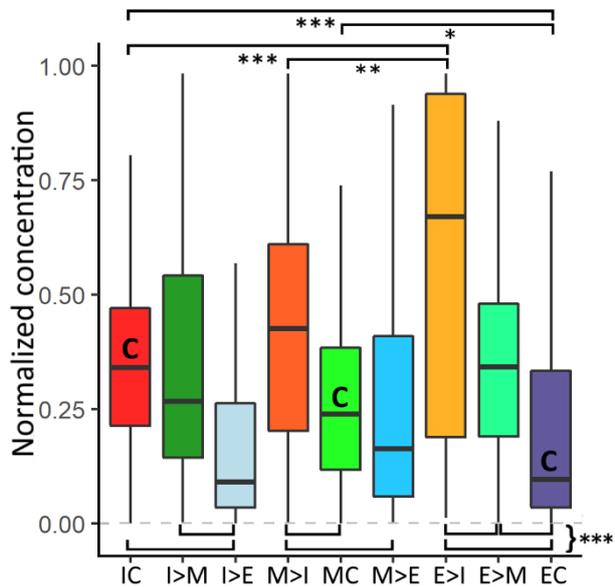
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363 For control (*i. e.* not transplanted) individuals of *B. neritina* sampled at the end of the experiment, all
364 MTEs excepted Cu and As showed significant differences between the three locations, with
365 systematically higher concentrations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

369 The normalized concentrations of all combined contaminants varied significantly between treatments
370 for *B. neritina* with a significant effect of 'origin' (two-way ANOVA; $p < 0.001$; $F = 7.23$), 'destination'
371 (two-way ANOVA; $p < 0.001$; $F = 77.86$) and their interaction (*i.e.* treatment; two-way ANOVA; $p <$
372 0.001 ; $F = 9.70$). The Tukey HSD test revealed that contaminant concentrations for the inner control IC
373 had significantly higher values than the two other controls ($p < 0.01$; Fig. 1). Higher contaminant
374 concentrations in *B. neritina* samples transplanted to the inner location were systematically observed,
375 compared to samples present at the entrance location at the end of the experiment, regardless of their
376 origin ($p < 0.001$; Fig. 1). It was only possible to observe an effect of origin for samples transplanted to
377 the inner location: E>I individuals had significantly higher normalized contaminant concentrations than
378 M>I and IC ($p < 0.01$; Fig. 1).

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

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



389
 390 **Fig. 1:** Boxplot of normalized concentrations of all contaminants in the bryozoan *Bugula neritina* according to
 391 their treatment. Treatments are labelled according to their origin followed (>) by their destination 2 months after
 392 transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated
 393 according to their destination (red = inner; green = middle; blue = entrance). Significant pairwise differences
 394 (Tukey's honest significance test of a 2-way Anova) are indicated by black brackets. *: $p < 0.05$; **: $p < 0.01$; ***:
 395 $p < 0.001$; no bracket: non-significant. All bottom brackets are ***. (n = 5)

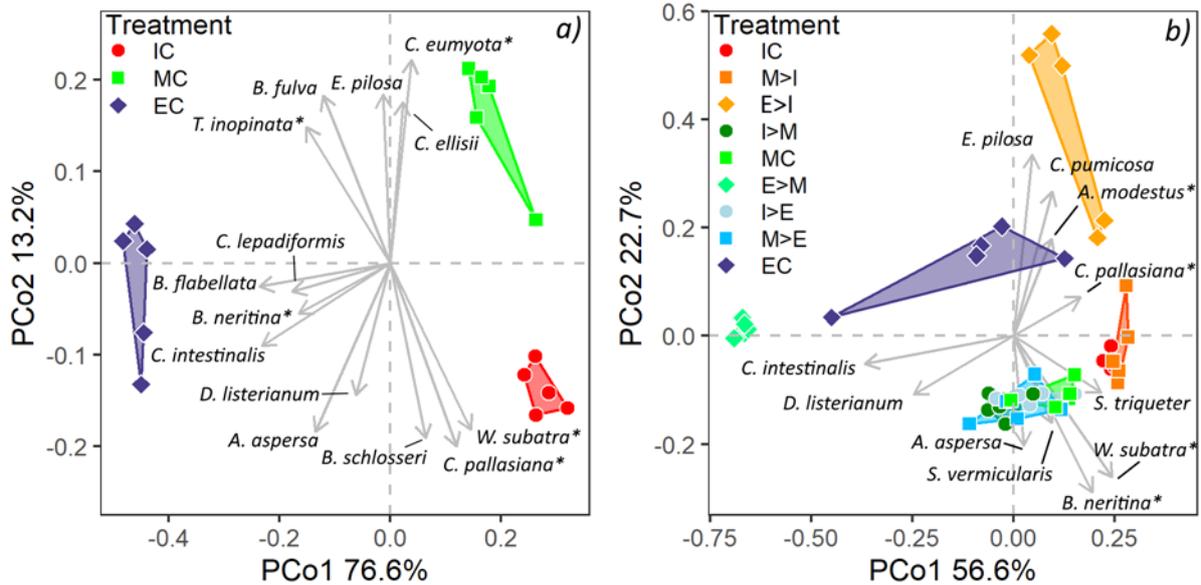
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397 3.2 Community

398 The photography-based community analysis did not show any difference among panels within each
 399 location before transplant (PERMANOVA; $p > 0.57$; $R^2 = 0.001$), thus excluding experimental artifacts
 400 due to panel selection. Panels sampled before transplant were significantly different according to their
 401 origin: IC vs MC (pairwise PERMANOVA; $p = 0.009$; $R^2 = 0.55$), MC vs EC (pairwise PERMANOVA; $p =$
 402 0.009 ; $R^2 = 0.87$), IC vs EC (pairwise PERMANOVA; $p = 0.009$; $R^2 = 0.89$). The PCoA (Fig. 2a) clearly
 403 discriminated EC from IC and MC along the first PCoA axis (76.6% of variability) while the second PCoA
 404 axis discriminated the two latter (13.2% of variability). The cover of several species varied between
 405 treatments before transplant. *C. intestinalis*, *A. aspersa* and *B. neritina* had the highest cover at the
 406 entrance location, while *W. subatra* had a lower cover at this location and higher at the inner location

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

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



428

429 **Fig. 2:** Principal Coordinate Analysis (PCoA) biplot of the community structure before transplant (a) and 2 months
 430 after transplant (b). Treatments are labelled according to their origin followed (>) by their destination after
 431 transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination) and indicated in the
 432 biplot according to their destination (red = inner; green = middle; blue = entrance) and origin (circle = inner,
 433 square = middle, diamond = entrance). Vector overlay indicates species with a positive correlation ($p < 0.05$; R^2
 434 > 0.2) with groups. Non-Indigenous species are indicated by an asterisk. Species list: *Ascidella aspersa*,
 435 *Austrominius modestus**, *Botryllus schlosseri*, *Bugula neritina**, *Bugulina flabellata*, *Bugulina fulva*, *Ciona*
 436 *intestinalis*, *Cellepore pumicosa*, *Clavelina lepadiformis*, *Corella eumyota**, *Cradoscrupocellaria ellisii*, *Cryptosula*
 437 *pallasiana**, *Diplosoma listerianum*, *Electra pilosa*, *Tricellaria inopinata**

438

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

444

445

446

447

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

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

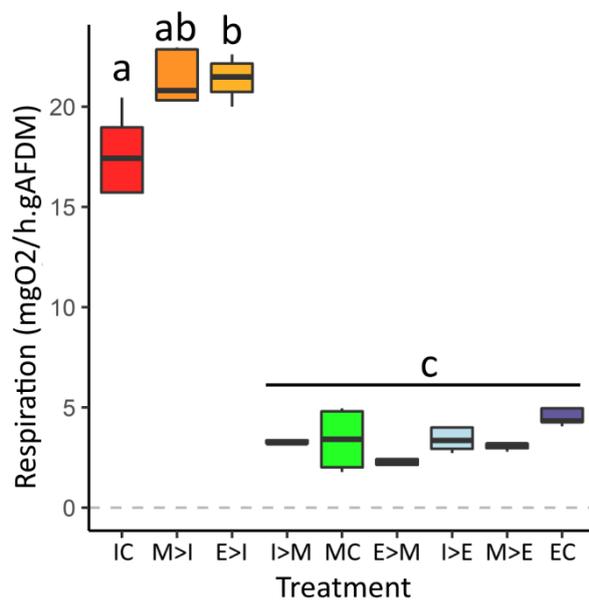
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455

456 3.3 Community respiration

457 Community respiration varied considerably among treatments with panels having the inner location
458 as destination (including IC) showing 3 to 5 times higher respiration rates than any other treatments
459 (Fig. 3). Respiration was significantly different between IC ($17.66 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{g}_{\text{AFDM}}^{-1}$) and the two other
460 controls (MC: $3.39 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{g}_{\text{AFDM}}^{-1}$; EC: $4.83 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{g}_{\text{AFDM}}^{-1}$; Tukey HSD; $p < 0.001$). M>I and E>I had
461 each higher respiration rates than treatments in other destinations (Tukey HSD; $p < 0.001$). However,
462 E>I respiration was even higher than the IC (E>I: $21.40 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{g}_{\text{AFDM}}^{-1}$; Tukey HSD; $p = 0.033$).



463

464 **Fig. 3:** Boxplot of the community respiration ($\text{mg O}_2 \cdot \text{h}^{-1} \cdot \text{g}_{\text{AFDM}}^{-1}$) for all treatments. Treatments are labelled
465 according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C:
466 controls *i.e.* identical Origin and Destination) and indicated according to their destination (red = inner; green =
467 middle; blue = entrance). Significant differences (Tukey HSD; $p < 0.05$) indicated by letter groups ($n = 5$).

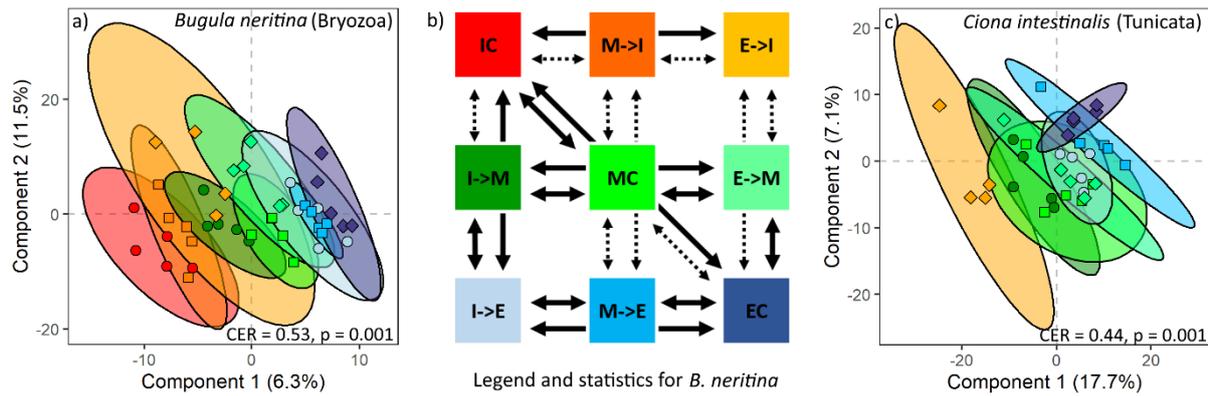
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469 3.4 Metabolome

470 The merged data matrix groups 597 features (LCMS¹⁺: 303, LCMS¹⁻: 282 and GCMS: 12) after
471 metabolome analysis for *B. neritina*, 522 features (LCMS¹⁺: 181, LCMS¹⁻: 344 and GCMS: 7) for *C.*
472 *intestinalis* and 428 features (LCMS¹⁺: 165, LCMS¹⁻: 171 and GCMS: 92) for *A. aspersa*. The PLS-DA of
473 the detected metabolome (LCMS¹⁺, LCMS¹⁻ and GCMS) of the three studied species was established

474 (Fig. 4; Sup. Fig. 4). For *B. neritina* and *C. intestinalis* the treatments are well separated on the
475 Component 1 axis, grouped by destination with the inner origin at the left side and the entrance origin
476 on the right for each destination group. Component 2 reflects the variability within each treatment.
477 The PLS-DA driven permutation Model Validation Analysis (MVA) revealed significant differences
478 among locations after transplant in the metabolomes of *B. neritina* (CER 'Classification Error Rate' =
479 0.53; $p < 0.001$; Fig. 4a) and of *C. intestinalis* (CER = 0.44; $p < 0.001$; Fig. 4c), but not of *A. aspersa*
480 (CER = 0.82; $p = 0.095$; Sup. Fig. 4). The pairwise MVA test showed a strong effect on the metabolome
481 of *B. neritina* with differences according to the treatments (Fig. 4b). The IC metabolome was
482 significantly different from the two other controls (MVA; $p < 0.05$). Several times, transplant resulted
483 in significantly different metabolomes compared to origin controls (destination effect) but also to
484 destination controls (origin effect). For *C. intestinalis*, it was not possible to recover samples from the
485 IC and M>I panels due to low biomass. Only the metabolome of E>I panels was significantly different
486 from all other treatments, except from M>I and MC ($p < 0.047$).

487 For *B. neritina* the 100 highest Variable of Importance in Projection (VIP) scores linked to the PLS-DA
488 were selected to putatively identify the metabolites linked to metabolome differentiation according
489 to treatments. Almost two third (59 %) of the VIPs were either sulfated or phosphated compounds.
490 Using LCMS² spectra to annotate metabolites, 46 of the 100 VIPs could be connected to a specific
491 chemical family (Tab. 4), including 16 glycerophosphoethanolamines (35%) and 13 sulfoethanolamines
492 (28%, a yet undescribed lipid class). While sulfoethanolamine VIPs had varying expression profiles,
493 associated to the inner or the entrance destinations, glycerophosphoethanolamine VIPs were more
494 prevalent in the MC and treatments including the entrance as a destination. Among other families, we
495 were also able to annotate some oxylipins, amines and purines. For *C. intestinalis* only some
496 glycerophospholipids were highlighted due to the absence of IC (Sup. Tab. 3). However, their pattern
497 was similar to glycerophosphoethanolamines in *B. neritina*.



498

499 **Fig. 4:** a) Partial Least Squares Discriminant Analysis (PLS-DA) of the metabolomes of *Bugula neritina* with b) its
 500 associated Statistical metrics for the pairwise comparisons and c) PLS-DA of the metabolome of *Ciona intestinalis*.
 501 Ellipses indicate confidence intervals of 0.95 for each treatment. Treatments are labelled according to their origin
 502 followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin
 503 and Destination) and indicated in the PLS-DA according to their destination (red = inner; green = middle; blue =
 504 entrance) and origin (circle= inner, square = middle, diamond = entrance). Full line arrow: pairwise MVA with p
 505 < 0.05. Dashed line arrow: non-significant. For both species one sample is missing in E>I while the treatments IC
 506 and M>I are completely absent for *C. intestinalis*. CER = Classification Error Rate with p-value after permanova
 507 test (permutations 10⁴).

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

527 **4. Discussion**

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

543

544 **4.1 Contamination in sediments and animals**

545 While no official common European guidelines for contamination in marine sediments do exist, we
546 could compare our results with two North American and two European guidelines. The harbor
547 sediments largely exceeded the various thresholds for some PAHs (chrysene, fluorene, fluoranthene
548 and phenanthrene) as well as for total PCBs (33 congeners). This may constitute an important stress
549 for local species, as the encountered concentrations would exceed a 50% probability of toxicity on
550 model amphipods exposed to the sediment (US EPA 2005). MTEs pollution presents higher global
551 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.

577 2008; Canning-Clode et al. 2011; Kenworthy et al. 2018b; Osborne and Poynton 2019). *Bugula neritina*
578 is particularly resistant to Cu, even compared to other introduced bryozoans like *Watersipora* spp.
579 commonly present in harbors (Piola and Johnston 2006a). Detoxification mechanisms may thus explain
580 why despite high Cu concentrations in sediments, *B. neritina* had overall low concentrations.

581 4.2 Community analyses

582 Previous studies have shown that pollution gradients may structure communities by inducing local
583 differences among them (Rondeau et al. in press; Je et al. 2004; Ryu et al. 2011; Kenworthy et al.
584 2018b). In the same marina as the present study, Kenworthy *et al.* (2018b) could correlate the high
585 but varying levels of some sediments contaminants (Cu, Zn, and hydrocarbons) at small spatial scales
586 (< 100 m) with heterogeneity in fouling communities of vertical pillars. While increasing the number
587 of tested contaminants, the present study showed similar results with significant differences
588 maintained throughout the whole duration of the experiment among communities from control panels
589 (Fig. 2). Such similar spatial patterns maintained over time might be caused ecological selection
590 (Vellend 2010). However, only an experimental approach such as reciprocal transplant may establish
591 a causal relationship between the environmental conditions, community structure and biological
592 functions (Osman and Whitlatch 2004; Cifuentes et al. 2010; Chang and Marshall 2016).

593 It was possible to observe a strong effect of the transplant on community structures. The global
594 PERMANOVA revealed a strong effect of origin, destination, and their interaction (Treatment). The
595 destination effect directly results from the transplant experiment as it reveals how location may select
596 the presence and/or abundance of certain taxa, driving similarities between communities at the inner
597 location. Here we confirmed differences in community composition between locations as previously
598 observed (Je et al. 2004; Ryu et al. 2011; Kenworthy et al. 2018b), but we also demonstrated a causal
599 relationship between location and community composition through the transplant experiment.
600 Predation or recruitment dynamics may contribute to the observed differences among communities.
601 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

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

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

653 4.3 Local adaptation is revealed at various organization levels and functions

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

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

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

683 Contamination in animal tissues may be an important measure to be accounted for, as it may impact
684 fitness through reproduction and survival (Piola and Johnston 2006a; b; Ruiz et al. 2011). In a recent
685 meta-analysis encompassing over 300 species, pollution has been demonstrated to be the anthropic
686 disturbance inducing the strongest phenotypic changes, potentially leading to rapid evolution
687 (Sanderson et al. 2022). Here, the contaminant composition of *B. neritina* (see Sup. Fig. 2, Tab. 1, 2 and
688 Sup. Tab 1) is mainly driven by MTE and PAH. Consequently, the EC (control at the least contaminated
689 location) had a significantly lower normalized concentration than the two other controls (Fig. 1), but
690 the transplant had a strong effect on *B. neritina* normalized contaminant concentrations: whatever
691 their origin, individuals transplanted towards the entrance showed the lowest concentrations, while
692 individuals transplanted to the inner location had systematically higher concentrations (Fig. 1).
693 Interestingly, it is also possible to observe a differential accumulation of contaminants among
694 individuals transplanted to the inner (most contaminated) location, with E>I individuals having a
695 significantly higher concentration than IC and M>I individuals (Fig. 1). This might be an indication for
696 local adaptation at very small scale (< 100 m) for this species, where individuals from less contaminated
697 locations (entrance) may have a lower detoxification activity (or higher contaminant uptake) compared
698 to their counterpart in contaminated locations (inner). There are many molecular and cellular
699 mechanisms used for detoxification which can be explained by genetic adaptation and differences in
700 expression regulation of detoxification pathways (Rainbow et al. 1990; Janssens et al. 2009). This
701 species has been shown to have differential resistance to Cu among populations (Piola and Johnston
702 2006b). Maternal effects seem to have a particularly strong effect on *B. neritina* facing spatial
703 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

781 5. Conclusion

782 The present results highlight strong small-scale spatial variation of contaminants in sediments and
783 animal samples. The community composition did not only vary in space in accordance to pollution as
784 it has already previously been observed (Kenworthy et al. 2018b), but it was causally linked to
785 environmental conditions as shown by the changes in community composition after transplant.
786 However, this destination effect is concomitantly associated with an effect of origin location indicating
787 that historical contingency and colonization succession may still play an equally crucial role. Our results
788 provide strong evidence for local adaptation at a very small spatial scale (< 100 m) since community
789 respiration, pollutant content of *B. neritina* and the metabolome of two species were impacted. We
790 expose a clear disadvantage for the 'foreign' condition for individuals transplanted from the entrance
791 to the inner, more polluted, location in community respiration, pollutant accumulation and
792 metabolome. As the considered distances are lower than the potential dispersal distances of the
793 studied species (Keough and Chernoff 1987; Svane and Havenhand 1993; Marshall and Keough 2003;
794 Shanks et al. 2003; Collin et al. 2013), the local adaptation observed here may even constitute a case
795 of microgeographic adaptation (Richardson et al. 2014). Dispersion and preferential settlement
796 observed in fouling species (Mckenzie et al. 2012; McDonald et al. 2014) might also accentuate local
797 differences if preferential settlement happens according to the individual phenotype (Bierne et al.
798 2003). While it might be possible that the studied species retained certain plastic traits from their
799 original location, time after transplant was long enough for transplanted communities to acclimate to
800 their new environment (one must consider that fouling communities are rather short-lived, mostly
801 months). We consider that the observed differences are thus likely profoundly anchored in the local
802 populations and may be regarded as local adaptation. The here observed results may thus indicate
803 that the disturbance gradient in marinas might constitute a staple for selecting pollutant-resistant
804 species and populations and cause local adaptation, which may explain why many NIS remain exclusive
805 to anthropic structures and marinas. However, in natural and urbanized habitats surrounding marinas,
806 pollution may occur in pulses due to wash off and water mixing events. Such pulse-pollution has

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

812

813 Acknowledgements

814 We would like to warmly thank Ports de Plaisance Brest and the Bureau du port de la Marina du
815 Château for providing access to the marina pontoons for experimentations. Special thanks to Aurélie
816 Dufour (MIO - UM 110) for participating in the analysis of MTE contaminants in sediment samples.
817 Special thanks also to Centre de Ressources Biologiques Marines (CRBM) and the Roscoff Aquarium
818 Services (RAS), specifically Gaëtan Schires and Sébastien Henry for providing access to aquaria facilities
819 and caring for the aquaria during species identification. Metabolomics analyses were conducted at
820 Chemical Ecology and Metabolomics facilities at IMBE (funded by ANR, French National Research
821 Agency, INEE the Institute of Ecology and Environment of the CNRS, the Total Foundation and the
822 French Sud PACA regional council). We also want to acknowledge Jean-Charles Martin (C2VN BIOMET
823 platform, Marseille) for creating in house metabolome filtering scripts for R. Funding for this project
824 has been provided through a PhD grant from the Sorbonne Université - Museum National d'Histoire
825 Naturelle (Ecole Doctorale 227). Financial support was also provided by the program 'InPor' of the
826 INEE-CNRS' PEPS EcoMob (PIs: C. Lejeusne and D. Davoult). The authors declare that no known
827 competing financial interests or personal relationships that could have appeared to influence the work
828 reported here exist. All applicable ethical guidelines were followed for our experimentations.

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1254

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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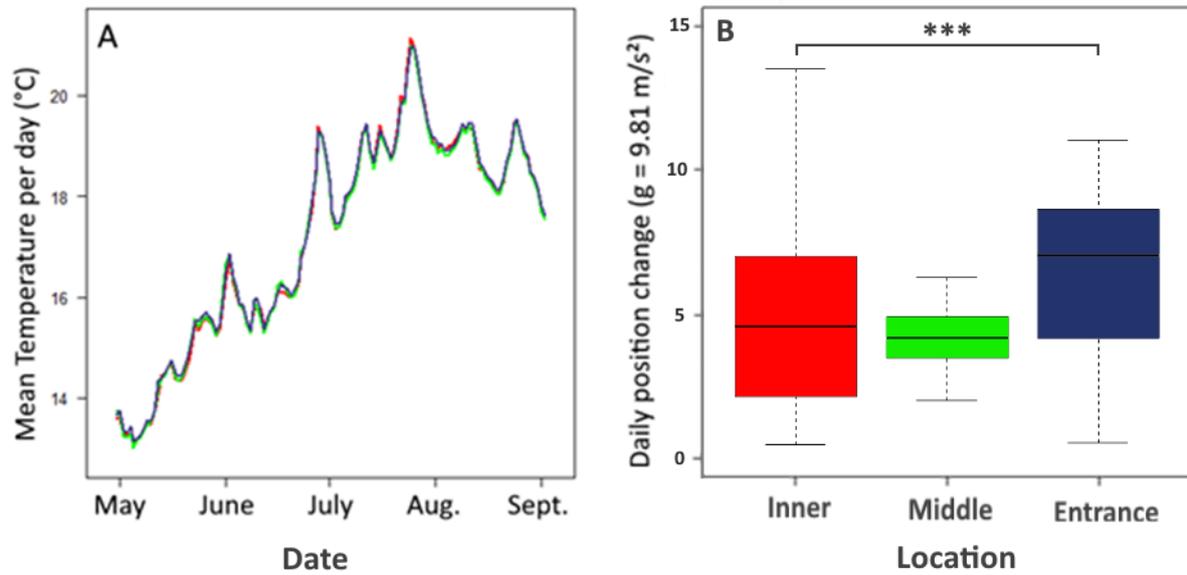
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Abstract. Urbanization of coastal habitats, of which harbors and marinas are the paragon, has led to various ecological paradigms about their functioning. Harbor infrastructures offer new hard substrata that are colonized by a wide variety of organisms (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.

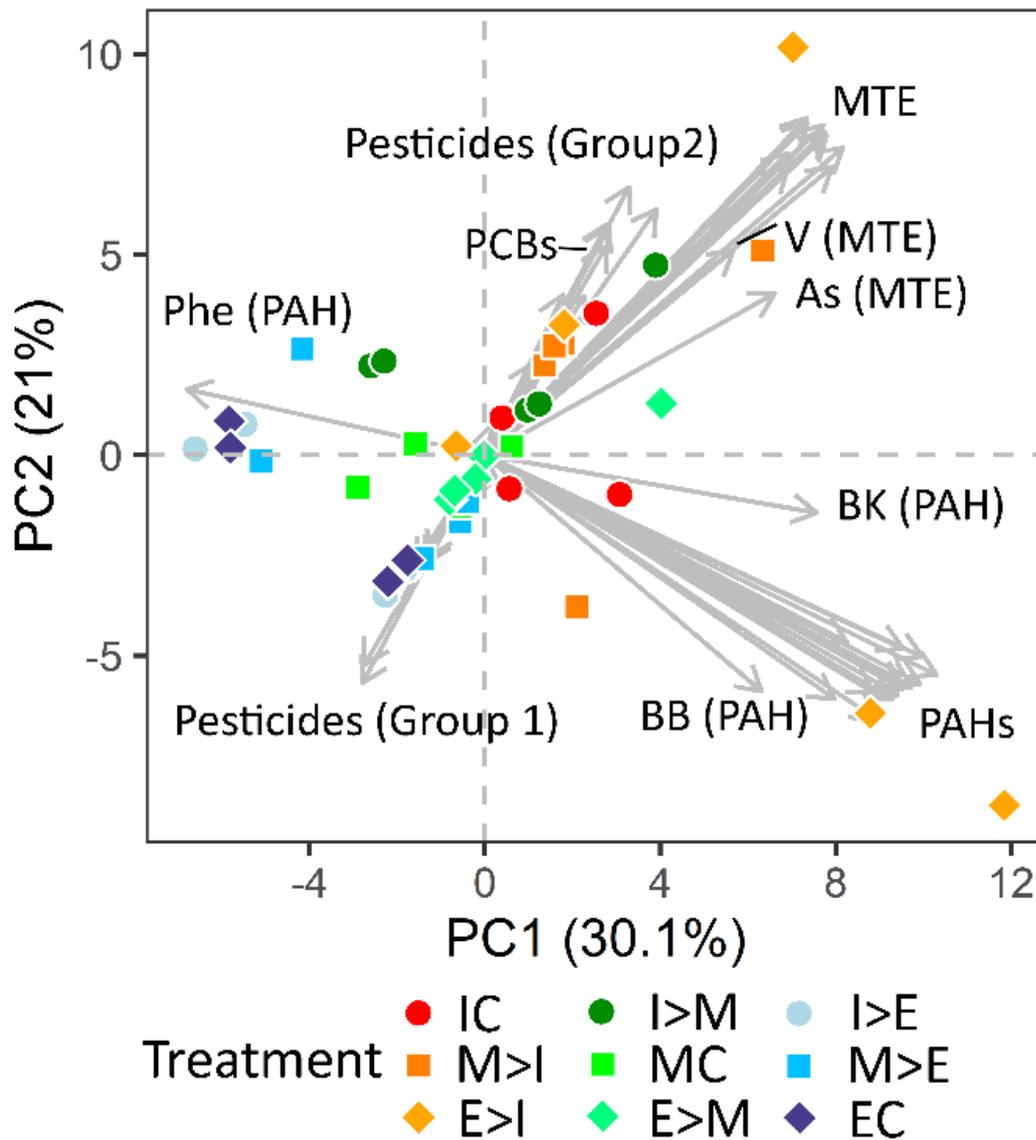
Keywords: local adaptation, fouling, pollution, metabolomics, respiration, marinas



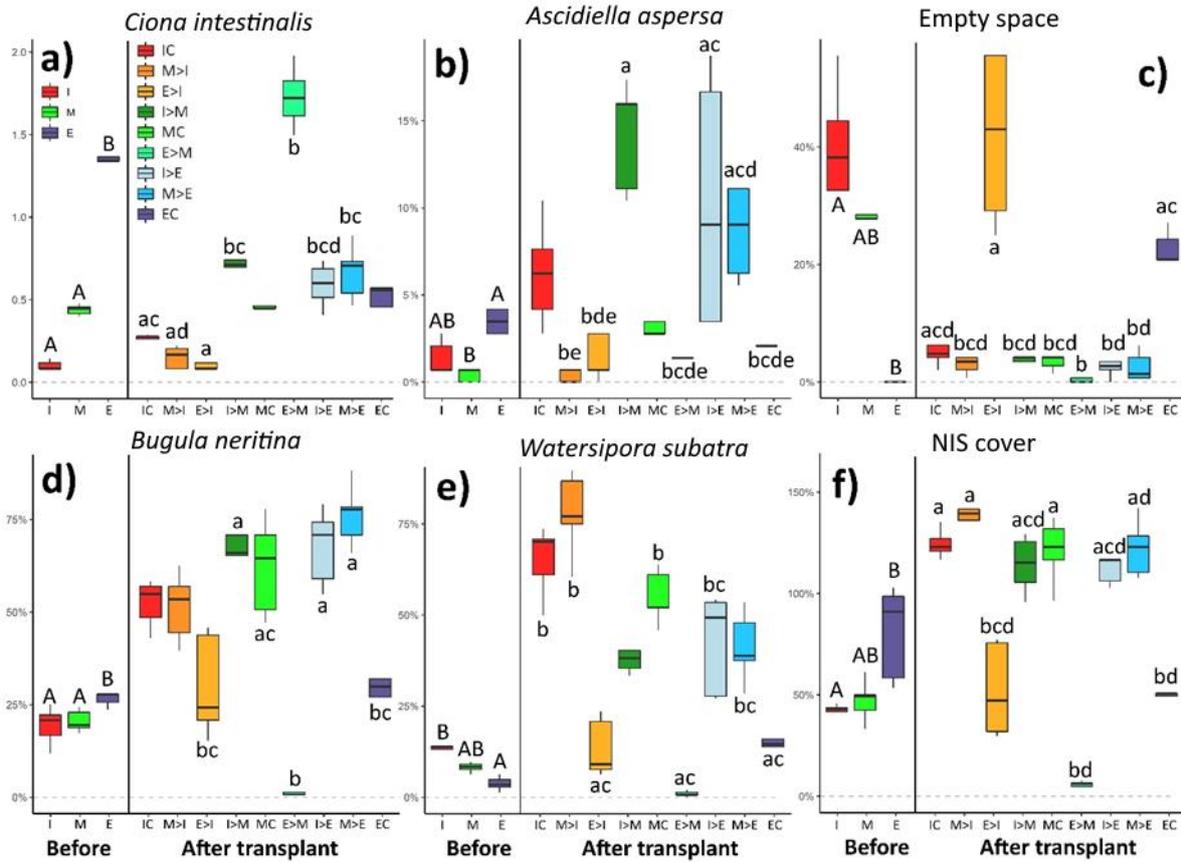
A. Fig. 1: Temperature curve (A) and boxplot of hydrodynamism (B) at the three locations. Red: Inner; Green: Middle; Blue: Entrance. For Temperature, the curves overlap although significant differences between pontoons were detected (LME, $t = 5.33$; $p < 0.05$). On a biological perspective, the differences were however negligible ($< 0.1^\circ\text{C}$). Daily position change in g ($9.81 \text{ m}\cdot\text{s}^{-2}$) 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 ($\mu\text{g}\cdot\text{kg}^{-1}$ = 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 States 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.

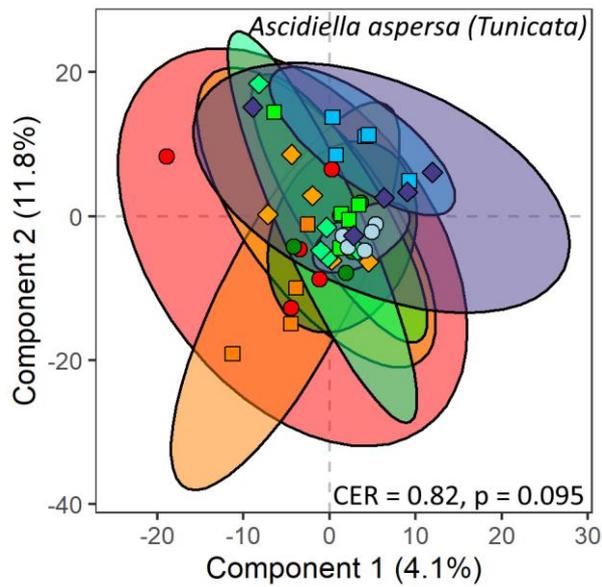
Pesticides ($\mu\text{g}\cdot\text{kg}^{-1}$)		Location mean \pm SD			K-W test	CCME		US EPA	
		Inner	Middle	Entrance		ISQG	PEL	T20	T50
Aldrin	Sediment	0.65 \pm 0.2	0.81 \pm 0.1	0.14 \pm 0.1	ns				
	<i>B. neritina</i>	1.31 \pm 0.1	0.89 \pm 0.1	1.76 \pm 0.3	*				
Cis_chlordane	Sediment	0.06 \pm 0.01	0.06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



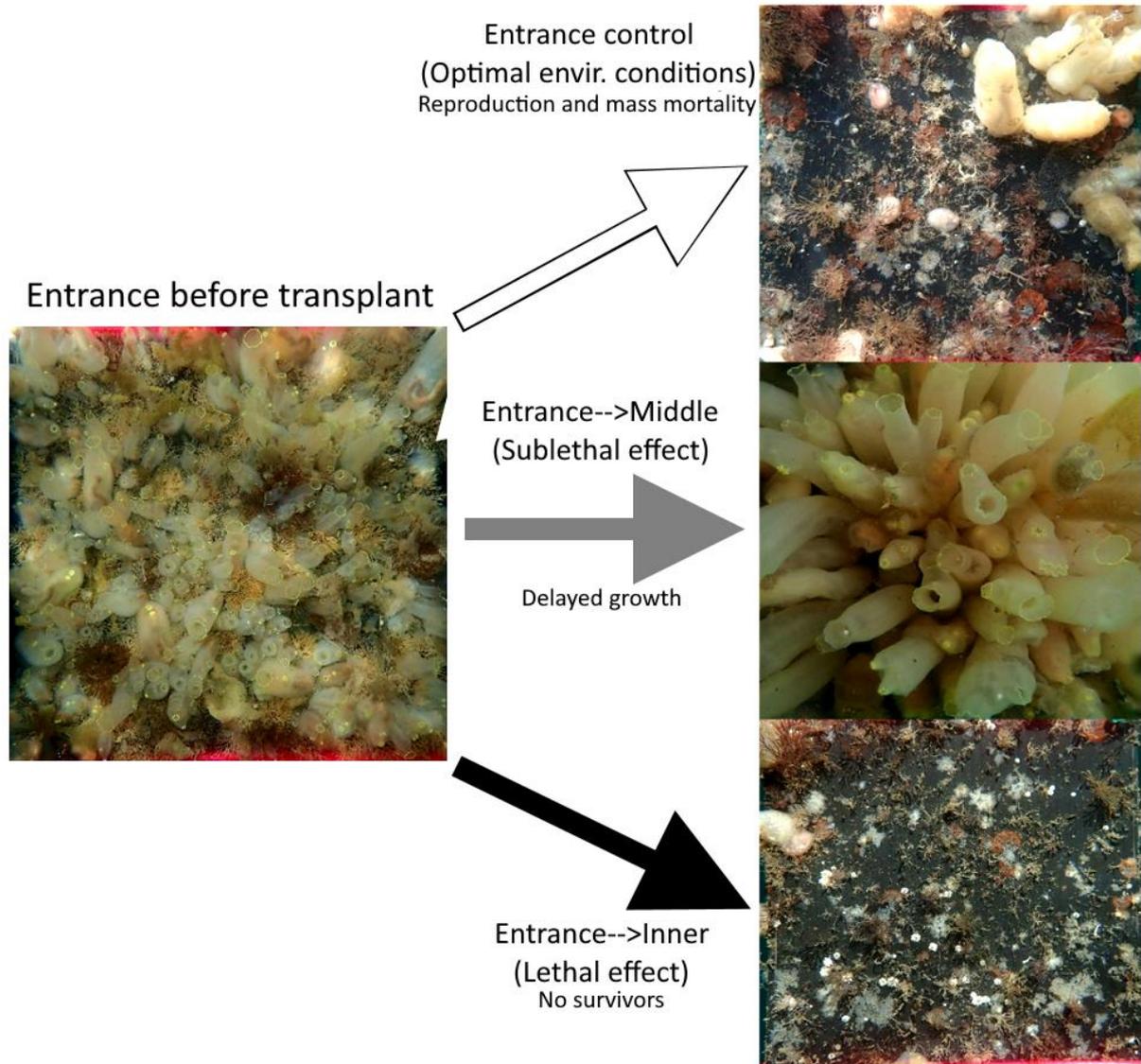
A. Fig. 2: Principal Component Analysis (PCA) biplot of contaminant concentrations in the bryozoan *Bugula neritina* according to their treatment. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* 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* (square-root-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.

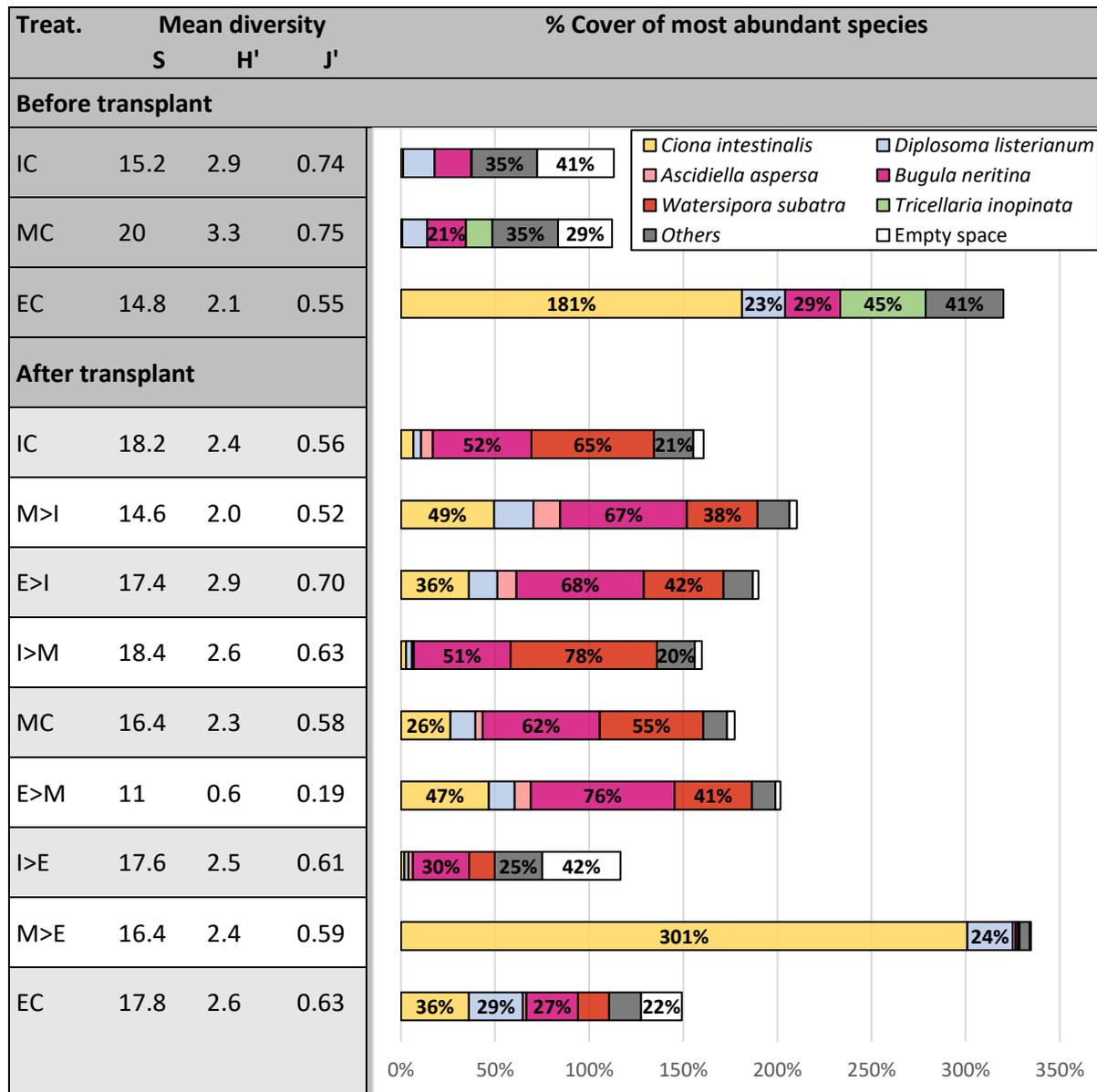


A. Fig. 4: Partial Least Squares Discriminant Analysis (PLS-DA) of the metabolome of *Ascidiella aspersa*. Ellipses indicate confidence intervals of 0.95 of each treatment (MVA test; $p = 0.095$, CER (Classification Error Rate) = 0.82). Treatments are labelled according to their origin followed (>) by 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).



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 *Ascidella 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 (CH₂Cl₂; 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 fractionated 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 H₂O. The extracts were transferred onto the cartridges, rinsed with 3 x 5 mL H₂O 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 LCMS¹⁺ and LCMS²⁺ (positive mode) and of 0.2 µL in LCMS¹⁻ and LCMS²⁻ (negative mode) was performed for *B. neritina*. For the other species, the injection volume was of 1 µL in LCMS¹⁺ and LCMS²⁺ and of 0.2 µL in LCMS¹⁻ and LCMS²⁻. LCMS parameters were set as follows: nebulizer gas, N₂ at 3.5 bars; dry gas, N₂ 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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