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1 **Getting used to the heat – Success of non-indigenous species facing**  
2 **climate change might depend on prior climate context.**

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3 **Abstract** - Climate change constitutes a major challenge for marine urban ecosystems and ocean  
4 warming will likely strongly affect local communities. Non-Indigenous Species (NIS) have been shown  
5 to often have higher heat resistance than natives, but studies investigating how forthcoming global  
6 warming might affect them in marine urban environments remain scarce, especially *in situ* studies.  
7 Here we used an *in situ* warming experiment in a NW Mediterranean (warm temperate) and a NE  
8 Atlantic (cold temperate) marina to see how global warming might affect recruited communities in the  
9 near future. In both marinas, warming resulted in significantly different community structure, lower  
10 biomass, and more empty space compared to control. However, while in the warm temperate marina,  
11 NIS showed an increased surface cover, it was reduced in the cold temperate one. Metabolomic  
12 analyses in the Atlantic marina revealed potential heat stress experienced by the introduced bryozoan  
13 *Bugula neritina* and identified a potential link between heat stress and the expression of a halogenated  
14 alkaloid, Caelestine A. The present results might indicate that global warming might not cause identical  
15 effects everywhere on the prevalence of NIS, and that present local climate may play a role in their  
16 response.

17 **Key Words** - Introduced species; Climate Change; *in situ* warming; marina; Hot Plates; Metabolomics

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32

## 33 Introduction

34 Climate change constitutes a major challenge for humanity and the situation has incited to strong  
35 political engagements to counter it (UN 2015; IPCC 2021). It is however unlikely that efforts suffice to  
36 limit global warming to +1.5°C, aimed for by the Paris agreement, and a scenario with a global  
37 temperature increase of +3.2°C seems most likely (Raftery et al. 2017). Associated to this, sea surface  
38 temperature is also projected to increase with climate change, with mean estimates ranging from  
39 +0.7°C to +3°C above preindustrial levels depending on the considered scenario (IPCC 2019). Climate  
40 change already poses a significant threat on natural ecosystems (Hanson and Weltzin 2000; Ayres and  
41 Lombardero 2000; Hughes et al. 2003; Lejeusne et al. 2010; Waldock et al. 2018; Lonhart et al. 2019),  
42 but as urban areas constitute islands of increased heat due to human activities and building materials,  
43 climate change effects may be even exacerbated (Chapman et al. 2017). These combined effects of  
44 climate and urbanization can be observed in terrestrial, aquatic and marine urban environments and  
45 are affecting species characteristics (Luo et al. 2006; Chapman et al. 2017; Brans et al. 2017; Todd et  
46 al. 2019). Understanding how climate change influences urban ecosystems, which are already  
47 subjected to cumulative anthropic impacts, constitutes a grand challenge of high priority (Borja et al.  
48 2020).

49 Climate change may substantially modify biological invasion patterns through niche modifications  
50 inducing new areas to become available for invasive species (Zhang et al. 2020; Vilizzi et al. 2021). NIS  
51 sometimes completely restructure the ecosystem leading to the loss of biodiversity and ecosystem  
52 services (Pejchar and Mooney 2009; Johnston et al. 2015; Walsh et al. 2016). Besides the long term  
53 warming, climate change is projected to further increase the frequency of extreme weather events  
54 such as heat waves which can cause mass mortalities and restructure communities in natural  
55 (Garrabou et al. 2009; Lejeusne et al. 2010; Collier and Waycott 2014) and in urban ecosystems (Smale  
56 et al. 2015; Castro et al. 2021). This effect may directly benefit NIS as many have been shown to have  
57 a higher temperature tolerance (Zerebecki and Sorte 2011; Lejeusne et al. 2014; Kenworthy et al.  
58 2018a; Gewing et al. 2019). Increased warming can also exacerbate the effect of pollution on local  
59 fauna by accelerating metabolism (Sokolova and Lannig 2008; Cabral et al. 2019; Todd et al. 2019). This  
60 may even further advantage NIS as they appear more tolerant to pollution as well (Piola and Johnston  
61 2008; Osborne and Poynton 2019). In the context of invasion, climate change might thus be a particular  
62 management challenge as it may increase the impact of NIS. For now, many NIS are established in their  
63 introduced range but are not considered invasive as they do not proliferate and do not cause adverse  
64 effects on the environment (Occhipinti-Ambrogi and Galil 2004; Blackburn et al. 2011; Ojaveer et al.  
65 2015). Yet some of these NIS may be sleeper species, which are currently impeded in their proliferation  
66 by environmental conditions and may become invasive if these conditions change due to climate

67 change (Bradley et al. 2018; Spear et al. 2021). Furthermore, climate exerts strong selective pressures  
68 on native and introduced populations and some species, including NIS, might rapidly adapt to climate  
69 change through genetically mediated differences in thermal tolerances (Brans et al. 2017; Sork 2018)  
70 or through plastic responses (Zerebecki and Sorte 2011; Sork 2018). Divergences in thermal tolerances  
71 of species from different geographical locations may be good indicators of their capacity to adapt to  
72 climate change (Sorte et al. 2011). It is thus crucial to foster the understanding of how climate change  
73 might impact marine urban communities, especially considering the important place the NIS occupy.

74 The effect of global warming and/or heatwaves on a variety of coastal ecosystems has most of the  
75 time been conducted through in aquaria and mesocosm studies (Sorte et al. 2010a; b; Smale et al.  
76 2015; Wahl et al. 2015; Pansch et al. 2018; Castro et al. 2021) but completely *in situ* experiments  
77 remain scarce (examples are Smale et al. 2011, 2017; Ashton et al. 2017), due to technical and  
78 experimental difficulties of *in situ* water warming. Marinas are the major model for studying marine  
79 urban communities, since they combine the different disturbances exerted by urbanization and have  
80 a high abundance of NIS, but they also offer logistic advantages making it possible to conduct *in situ*  
81 water warming experiments in an anthropogenic context.

82 In the present study we thus aim to assess the *in situ* effect of +3°C sea surface warming (RCP8.5; IPCC  
83 2019) in two marinas, one in the NW Mediterranean and one in the NE Atlantic. We deployed indirectly  
84 heated hot plates in open systems in two marinas and assessed differences in sessile community  
85 characteristics from not heated control panels. We also focused on the metabolism of a common  
86 marina species (reported as NIS in both surveyed marinas) to see if it was affected under increased  
87 heat. The observed results could help to make predictions about the effect of climate change on these  
88 two marinas, especially in regard to the prevalence of NIS, which are supposed to be advantaged by  
89 increased heat.

## 90 **Materials and Methods**

### 91 ***Study sites***

92 The two sites selected for our study were the Marina du Chateau in Brest (henceforth “Chateau”;  
93 Finistere, France; 48°22'45.4"N, 4°29'21.2"W) and the Port de Carry-le-Rouet (henceforth “Carry”;  
94 Bouches-du-Rhône, France; 43°19'47.0"N, 5°09'09.5"E). Both marinas have similar boating capacities  
95 (650 and 590 berths respectively), are semi-enclosed, and subject to anthropic impacts due to their  
96 position in their urban area and water runoffs at the inner parts of the marinas. We deployed  
97 experimental setups at the entrance of both marinas to avoid a synergetic and confounding effect of  
98 strong pollution in the inner parts of marinas (Je et al. 2004; Kenworthy et al. 2018b).

99 **Experimental setup**

100 For each marina we deployed a structure with 10 settlement panels (Correx Polyethylene, 20 cm x 20  
101 cm; Fig. 1). These recruitment panels were randomly assigned to be either control panels (5 per  
102 marina) or being constantly heated at +3°C above the recorded ambient water temperature (5 per  
103 marina). The experiment lasted 50 days in both marinas and started in mid-August 2020 in Carry and  
104 early September for Chateau. To reach a stable water temperature difference with ambient water, the  
105 settlement panels were placed at the top of transparent bottom opened acryl cylinders. Water column  
106 warming of the cylinders was achieved through 200W aquaria resistors (approx. 20 cm away from the  
107 panels) regulated with a built-in computer (Raspberry Pi3+) coupled to thermometers (DS18B20)  
108 measuring the outside water temperature (Fig. 1, Sup. Fig. 1). The water temperature around the  
109 heating elements and directly around the panels was regularly monitored to avoid overheating.  
110 Passthroughs at the top of the cylinders aimed to increase connectivity with the surrounding water  
111 and to avoid excessive stratification of the water within the cylinder.



112  
113 *Fig. 1: in situ warming structure deployed in the marinas. Photo credit: Wilfried Thomas (CNRS Station Biologique*  
114 *de Roscoff)*

115 **Fauna identification and community analysis**

116 At the end of the experiment all panels were transported to the laboratory where they were  
117 maintained in open circuit aquaria until identification by a single observer in less than 36 h. A surface  
118 of 15 cm x 15 cm was analyzed by superimposing a 100-point grid allowing for a resolution of more  
119 than 0.4 points per cm<sup>2</sup> necessary for a reliable estimation of the surface cover (Taormina et al. 2020).  
120 At each intersection of the grid all living individuals of any species were counted. Species present on a  
121 panel that did not fall within an intersection were counted with 1% cover. Identification went to the  
122 lowest possible taxonomical level (Zibrowius 1968; Hayward and Ryland 1979, 1985, 1995, 1998;  
123 Bianchi 1981; Riedl 1983; Zabala and Maluquer 1988; Brunetti and Mastrototaro 2017). After analysis,

124 the panels were scraped, and biomass was dried at 60°C for one week, then weighted. To determine  
125 ash free dry mass (AFDM) the dry communities were burned at 520°C for 6 h.

126 The community matrix has been analyzed using the 'vegan' R package (version 2.4-6; Oksanen et al.  
127 2018). The Shannon ( $H'$ ) and Simpson ( $\lambda$ ) diversity indices as well as Pielou ( $J'$ ) evenness and species  
128 richness were assessed for each panel. They were compared between the control and heated  
129 treatment with an ANOVA. A Poisson GLM was applied for species richness since it constitutes count  
130 data. The community matrix was transformed into a Bray-Curtis dissimilarity matrix and multivariate  
131 homogeneity of groups was verified with the 'betadisper' function. A Principal Coordinate Analysis  
132 (PCoA) was used to visualize the community of both treatments for the two marinas. A subsequent  
133 PERMANOVA ( $10^5$  perm.) was used to assess for significant differences between treatments of each  
134 marina. To identify the species contributing to the differences between treatments a SIMPER analysis  
135 followed by an Multipattern analysis using the 'indicSpecies' package was conducted ( $10^4$  perm.; ver.  
136 1.7.1; Cáceres et al. 2011; De Cáceres 2013). An ANOVA was used on the AFDM to determine if  
137 significant differences were present between treatments for each marina. The cover occupied by  
138 empty space without any species and the cover exerted by NIS, was analyzed via a Poisson glm since  
139 they originated from count data (One count = 1% cover).

#### 140 ***Metabolome identification and analysis***

141 The invasive bryozoan *Bugula neritina* was used for metabolomic analyses, as it usually occurs in both  
142 marinas due to its worldwide introduction and having been previously studied in the Chateau marina  
143 including for metabolomics (Gauff et al. in prep a). Unfortunately, biomass in the experiment from the  
144 Carry marina was too low for metabolomic sampling, so analyses were only conducted in the Chateau  
145 marina. For each panel, one large colony of *B. neritina* was sampled. Samples were immediately frozen  
146 in dry ice, then stored at -80°C, freeze dried and ground to dust. The total metabolome was extracted  
147 out of 5 mg of dry powder with 3 times 1 mL MeOH/DCM (CH<sub>2</sub>Cl<sub>2</sub>; 1:1) and ultrasonication at room  
148 temperature during 2 min. Supernatants were filtered on PTFE syringe filters (13 mm, 0.22 μm,  
149 Restek®). Two samples (one of each treatment) were excluded due to strong contamination. Before  
150 injection into Liquid Chromatography Mass Spectrometry (LC-MS) the samples were resuspended in  
151 500 μL MeOH and a pool sample was created by mixing 30 μL of each. Two blank samples, which were  
152 submitted to the entirety of the extraction process were additionally produced. LC-MS were performed  
153 with UHPLC (Dionex Ultimate 3000 with RS Pump, autosampler, thermostat column and UV diode  
154 array, Thermo Scientific®) coupled to a quadrupole Time of Flight spectrometer (qToF) with an ESI  
155 source (Impact II, Bruker Daltonics®). We conducted UHPLC separation on an Acclaim™ RSLC 120C18  
156 column (150 mm x 2.1 mm, 2.2 μm, ThermoScientific®) at 40°C at 0.5 mL min<sup>-1</sup> elution rate. Water

157 (Polar, LC/MS grade, Carlo Erba®) with 0.1% formic acid (A) and acetonitrile (Apolar, LC/MS grade) with  
158 0.1% formic acid (B) were used as chromatographic solvents. Chromatography cycles were set as  
159 follows: 1) mix of 5:95 of A:B during 2 min, 2) linear increase up to 100% B during 8 min followed by  
160 100% B during 4 min, 3) return to initial conditions (5:95, A:B) for 3 min (17 min total). We injected 10  
161  $\mu\text{L}$  in MS1+ and MS2+ with  $\text{N}_2$  at 3.5 bars as nebulizer gas,  $\text{N}_2$  at  $12 \text{ L min}^{-1}$  as dry gas, capillary temp.  
162 at  $200^\circ\text{C}$  and voltage at 3500 V for a scan from 50 to 1200 mass units at 2 Hz. A spectrometer  
163 calibration with a formate:acetate solution was performed before each sample and the pool sample  
164 was injected every 4 samples to correct for source drift. Blank samples and pure solvent were injected  
165 to filter for ions linked to the extraction process.

166 LC-MS profiles were automatically recalibrated with the internal calibration in 'Bruker Compass  
167 DataAnalysis' (version 4.3) resulting in a  $m/z$  precision of 2 ppm. The netCDF data was processed with  
168 the 'XCMS' R package (version 3.12; Smith et al. 2006) with peak picking for the detection of features,  
169 obiwarp retention time correction, feature grouping (time frame 5 s) and peak filling. Ions were  
170 manually normalized in Microsoft Excel (Van Der Kloet et al. 2009) and were filtered to exclude ions  
171 originating from the blanks, based on signal/noise ratio ( $S/N = 10$ ), ions with high variability in the pool  
172 sample ( $> 0.2$ ) and ions that were auto-correlated ( $> 0.8$ ). To increase relevancy, we excluded the first  
173 100 seconds of chromatography of our analysis.

174 Metabolome analyses were mainly performed in 'Metaboanalyst' (Xia et al. 2009). Our matrix had less  
175 than 5000 features and was log transformed and auto scaled. A Principal Correspondence Analysis  
176 (PCA) was used to visualize our sample structure. A PPLS-DA driven permutation Model Validation  
177 Analysis (MVA) ( $10^5$  perm., 6 components) using the 'RVAideMemoire' package in R (version 0.9-79;  
178 Hervé 2021) tested for statistical differences between the treatments. A Volcano plot from  
179 'Metaboanalyst' identified features with a significant foldchange (t-Test,  $p < 0.05$ ;  $FC > 1.5$ ). These  
180 molecules were analyzed with fragmentation mass spectrometry (MS2) spectra. Molecular networking  
181 via Global Natural Products Social Molecular Networking (GNPS; Wang et al. 2016) and visualized in  
182 'cytoscape' (version 3.7.2) helped ion identification.

183 Molecules of particular interest, requiring further confirmation upon identification were compared  
184 against a commercially produced standards (Ambinter®, Greenpharma) by injecting the sample with  
185 the highest intensity and the commercial extract in a short injection series following the same  
186 parameters as described earlier.

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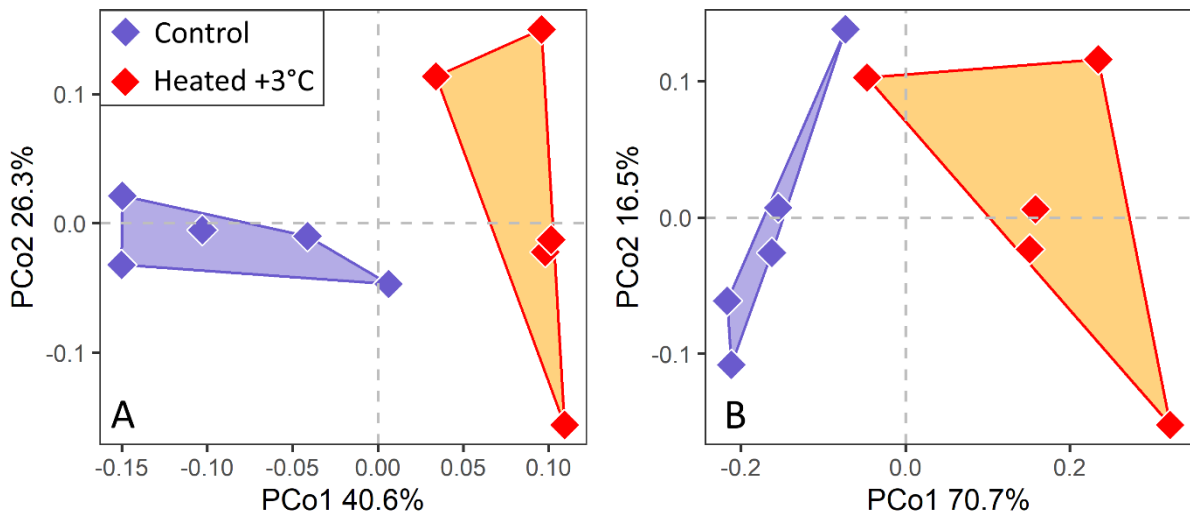
189 **Results**

190 **Community structure**

191 None of the diversity indices had significant differences between control and heated panels in any  
192 marina (Carry:  $H' J' \lambda$ , ANOVA,  $|R^2| < 0.27$ ,  $p > 0.07$ ; S, Poisson glm,  $z = -0.52$ ,  $p = 0.62$ ; Chateau:  $H' J' \lambda$ ,  
193 ANOVA,  $|R^2| < 0.12$ ;  $p > 0.33$ ; S, Poisson glm,  $z = 0.25$ ,  $p = 0.81$ ). The species list for each marina can  
194 be found in Sup. Tab. 1. On the PCoA of the community structure (Fig. 2), panels were distributed  
195 according to their treatment (Control vs Heated) on the PCo1 axis, explaining 40.6% of the variability  
196 between samples for Carry (Fig. 2 A) and 70.7% for Chateau (Fig. 2 B). The PCo2 axis ( $< 26.3\%$ )  
197 corresponded to intra treatment variability. For both marinas, significant effects of the warming were  
198 observable on the communities (PERMANOVA; Carry:  $R^2 = 0.34$ ,  $p = 0.008$ ; Chateau:  $R^2 = 0.54$ ,  $p =$   
199  $0.008$ ). The SIMPER analysis revealed that in both cases, 5 species contributed to more than 70% of  
200 the contrast between the treatments (over 85% for Chateau; Fig. 3; Tab. 1). At the Carry marina, only  
201 the bryozoan *Aetea sica* has been identified as indicator species for the control (Tab. 1). In the Château  
202 marina however, most of the contributors identified by SIMPER analysis were also indicator species of  
203 the control treatment (Tab. 1). For this marina two additional species were identified as indicator  
204 species for the heated treatment: the bryozoan *Cryptosula pallasiana* and the annelid *Spirobranchus*  
205 *triqueter* (Tab. 1).

206

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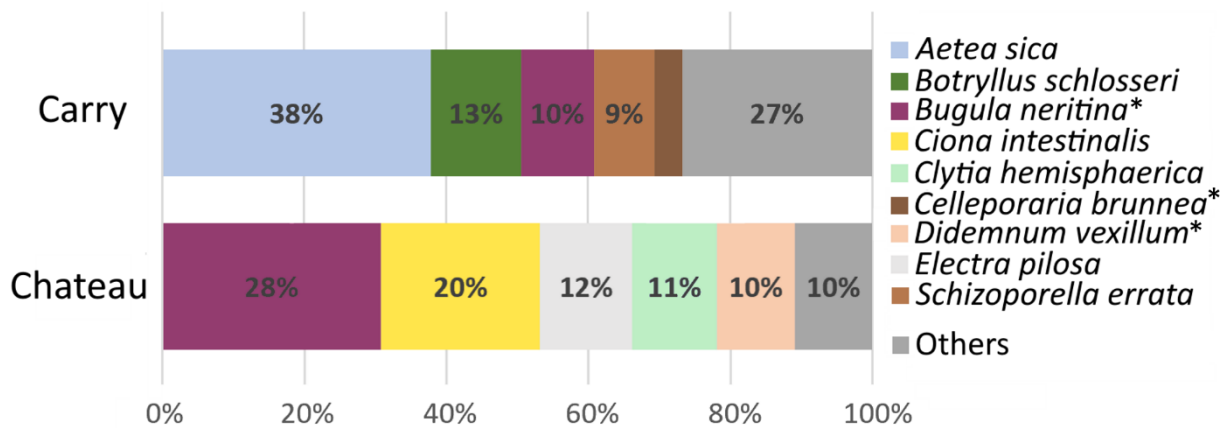


208

209 *Fig. 2: Principal Coordinate Analysis (PCoA) of the control and heated (+3°C) communities in the A) Carry-le-Rouet*  
210 *Marina (Mediterranean) and in B) Marina du Château (Atlantic). Significant differences in community structure:*  
211 *PERMANOVA: Carry ( $R^2 = 0.34$ ,  $p = 0.008$ ); Chateau ( $R^2 = 0.54$ ,  $p = 0.008$ )*



212



213

214 Fig. 3: Cumulated contribution of different species to the contrast between the control and heated (+3°C)  
 215 communities in the Carry-le-Rouet and Chateau marinas (SIMPER analysis). Species with an asterisk are non-  
 216 indigenous species.

217 Tab. 1: Species most contributing to the contrast between the control and heated (+3°C) communities and  
 218 indicator species for the treatments. Species in bold with an asterisk are non-indigenous species. SIMPER analysis  
 219 score indicates the percentage a species contributes to the contrast between treatments (see also Fig. 3). The  
 220 direction of the warming effect on the percent cover occupied by these species is indicated. Indicator species for  
 221 each treatment identified through Multipattern analysis are indicated with their association statistic (Stat.) and  
 222 the p-value. SIMPER scores in parentheses are scores that are not part of the most important contributors.

Carry-le-Rouet						
Species	SIMPER Score	Effect of warming	Indicator species control		Indicator species heated	
			Stat.	p-value	Stat.	p-value
<i>Aetea sica</i>	38%	↘	0.76	0.008		
<i>Botryllus schlosseri</i>	13%	↘				
<b><i>Bugula neritina*</i></b>	10%	↗				
<i>Schizoporella errata</i>	9%	↘				
<b><i>Celleporaria brunnea*</i></b>	4%	↗				

Chateau Marina						
Species	SIMPER Score	Effect of warming	Indicator species control		Indicator species heated	
			Stat.	p-value	Stat.	p-value
<b><i>Bugula neritina*</i></b>	28%	↘	0.78	0.015		
<i>Ciona intestinalis</i>	20%	↘	0.89	0.007		
<i>Electra pilosa</i>	12%	↘	0.84	0.040		
<i>Clytia hemisphaerica</i>	11%	↗				
<b><i>Didemnum vexillum*</i></b>	10%	↘	0.90	0.015		
<i>Spirobranchus triqueter</i>	(4%)	↗			0.90	0.047
<b><i>Cryptosula pallasiana*</i></b>	(3%)	↗			0.91	0.033

223

224

225 Ash free dry mass (AFDM) varied significantly between treatments for both marinas (ANOVA,  $R^2 > 0.56$ ,  
 226  $p < 0.005$ ; Fig. 4) with lower biomass in the heated treatment. Accordingly, empty space was  
 227 significantly higher in the heated treatment for both marinas (Poisson glm,  $z > 2.28$ ,  $p < 0.022$ ; Fig. 4 A  
 228 B). The absolute cover exerted by NIS as well as the ratio of NIS cover on total cover (proportional  
 229 cover) was higher in the heated treatment for Carry-le-Rouet (Poisson glm,  $z = 2.149$ ,  $p = 0.0317$ ; Fig.  
 230 4 C; ANOVA,  $R^2 = 0.29$ ,  $p$  value = 0.063). In the Chateau marina the absolute cover was significantly  
 231 lower in the heated treatment while the proportional cover remained unaffected (Poisson glm,  $z = -$   
 232 5.36,  $p < 0.001$ ; Fig. 4 D; ANOVA, NS).

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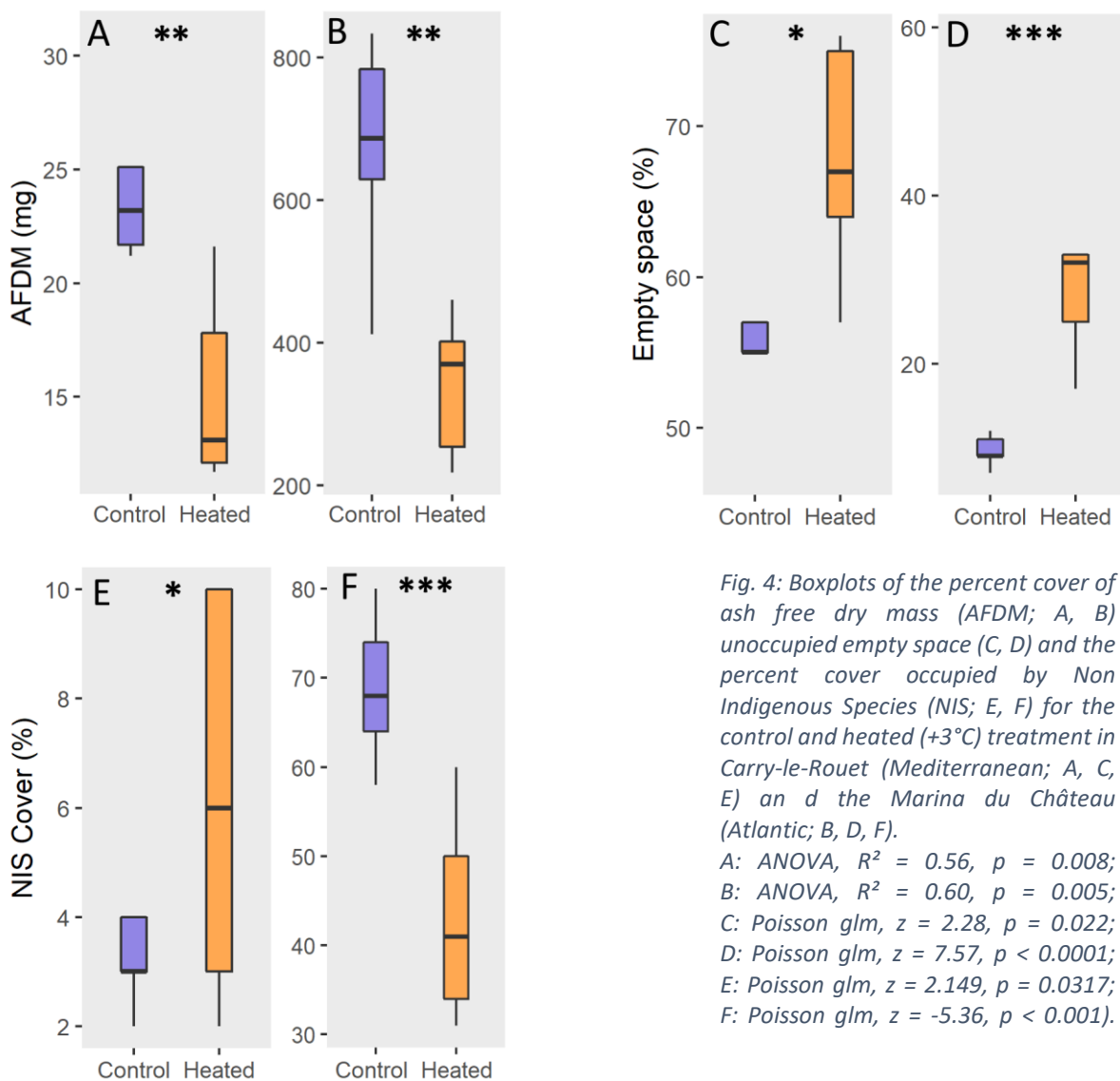


Fig. 4: Boxplots of the percent cover of ash free dry mass (AFDM; A, B) unoccupied empty space (C, D) and the percent cover occupied by Non Indigenous Species (NIS; E, F) for the control and heated (+3°C) treatment in Carry-le-Rouet (Mediterranean; A, C, E) and the Marina du Château (Atlantic; B, D, F).

A: ANOVA,  $R^2 = 0.56$ ,  $p = 0.008$ ;  
 B: ANOVA,  $R^2 = 0.60$ ,  $p = 0.005$ ;  
 C: Poisson glm,  $z = 2.28$ ,  $p = 0.022$ ;  
 D: Poisson glm,  $z = 7.57$ ,  $p < 0.0001$ ;  
 E: Poisson glm,  $z = 2.149$ ,  $p = 0.0317$ ;  
 F: Poisson glm,  $z = -5.36$ ,  $p < 0.001$ ).

235

236

237 **Metabolome analysis**

238 The metabolome of *Bugula neritina* showed a marginally significant difference between the control  
 239 and the heated treatment (Fig. 5; MVA: CER = 0.181, p-value = 0.052). A total of 12 molecules were  
 240 found to vary significantly between treatments. Seven had a significant 1.5-fold under expression while  
 241 5 had a significant 1.5-fold overexpression (t-Test,  $p < 0.05$ ; Fig. 6). From these molecules, we were  
 242 able to identify 6 (Tab. 2). A molecule identified as a Bromo-Oxo-Quinoline-Carboxylic Acid (BQA) had  
 243 a fragmentation pattern (Sup. Fig. 2) compatible with a previously described molecule (Caelestine A;  
 244 7-bromo-4-oxo-1,4-dihydroquinoline-2-carboxylic acid; Yin et al. 2010) and was thus compared against  
 245 a commercially available Caelestine A. Extracted ion chromatogram and fragmentation pattern were  
 246 identical (Sup. Fig. 3).

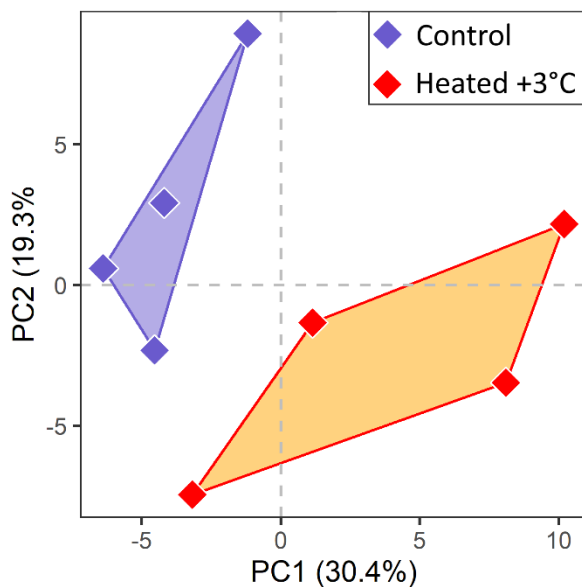
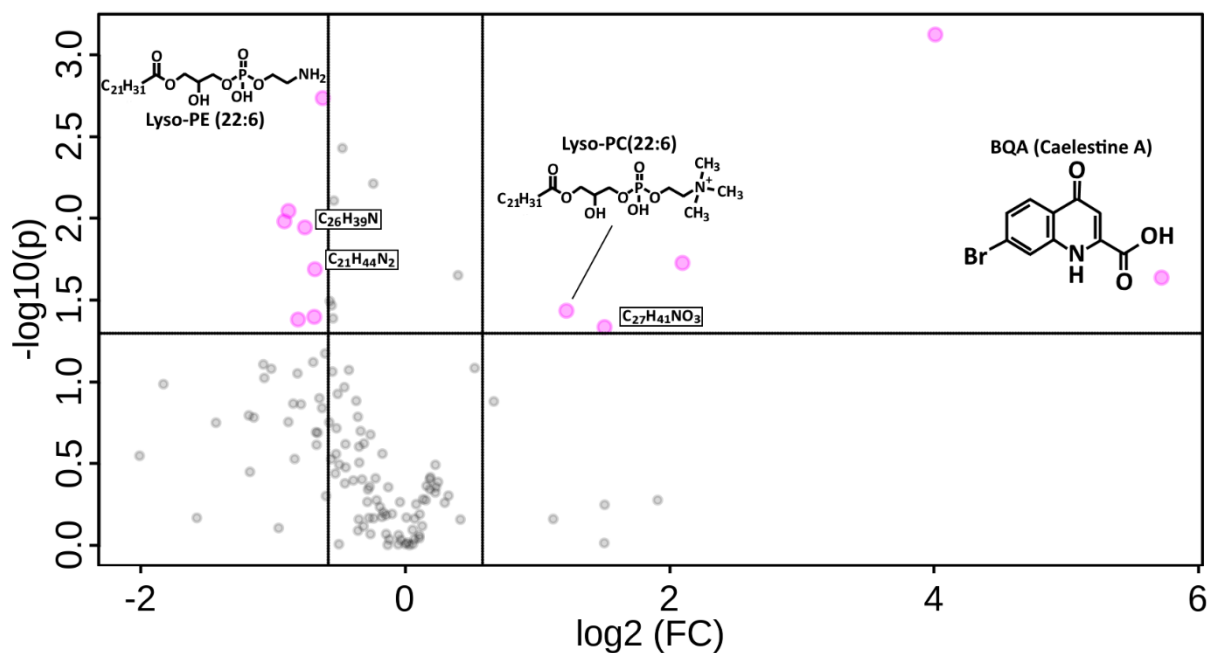


Fig. 5: Principal Component Analysis (PCA) of the metabolome of *Bugula neritina* for the control and heated (+3°C) treatments in the Chateau marina (MVA: CER = 0.181, p-value = 0.052).



258 Fig. 6: Volcano plot showing molecules of the metabolome of *Bugula neritina* in the Chateau marina (Atlantic)  
 259 with a significant (*t*-test;  $p < 0.05$ ) fold change ( $FC > 1.5$ ) in the heated (+3°C) treatment compared to control  
 260 (indicated by larger dots on plot). Left: underexpressed molecules compared to control; Right: overexpressed  
 261 molecules compared to control. The x-intercepts correspond to -1.5 and 1.5 FC. The y-intercept corresponds to a  
 262 *p*-value of 0.05. Molecules from Tab. 2 indicated.

263 Tab. 2: Identified molecules with a significant fold change (FC) between heated vs control treatments in *Bugula*  
 264 *neritina* in the Chateau marina. Identification confidence levels are indicated (Schymanski et al. 2014): 5 Exact  
 265 mass (with tentative formula), 4 Unequivocal molecular formula, 3 Tentative candidate (structure), 2b Probable  
 266 structure by diagnostic evidence, 1 Confirmed structure by reference standard.

Molecule	Formula	m/z	rt	FC	p.value	Confidence level	
C <sub>21</sub> H <sub>44</sub> N <sub>2</sub>	C <sub>21</sub> H <sub>44</sub> N <sub>2</sub>	325.36	538	↘	0,54	0,01	5
C <sub>26</sub> H <sub>39</sub> N	C <sub>26</sub> H <sub>39</sub> N	366.32	648	↘	0,59	0,01	4
Lyso-PE (22:6)	C <sub>27</sub> H <sub>44</sub> NO <sub>7</sub> P	526.29	612	↘	0,65	< 0.001	2b
Lyso-PC (22:6)	C <sub>30</sub> H <sub>50</sub> NO <sub>7</sub> P	568.34	623	↗	2,33	0,04	2b
C <sub>27</sub> H <sub>41</sub> NO <sub>3</sub>	C <sub>27</sub> H <sub>41</sub> NO <sub>3</sub>	428.32	769	↗	2,84	0,05	5 to 4
Caelestine A	C <sub>10</sub> H <sub>6</sub> BrNO <sub>3</sub>	267.96	394	↗	52,73	0,02	1

267

## 268 Discussion

269 The present results indicate that long-term warming due to climate change may affect marina  
 270 communities and the metabolism of certain species. We chose to simulate *in situ* a seawater warming  
 271 of +3°C compared to the control in accordance with the mean estimate of global surface temperature  
 272 increase projected in RCP8.5 (IPCC 2019). Simulated warming in mesocosms (Sorte et al. 2010b) and  
 273 *in situ* (Smale et al. 2011, 2017; Ashton et al. 2017) has been shown to strongly impact communities  
 274 favoring opportunistic species and NIS. In accordance with these previous observations, our present  
 275 case revealed significant differences in the recruited communities between heated and control  
 276 treatments in both marinas, which was associated to lower biomass (AFDM) and higher empty space  
 277 in the heated treatment. At Carry-le-Rouet (Carry, Mediterranean Sea), this coincided with higher  
 278 absolute and proportional abundance of non-indigenous species in the heated treatment. In the  
 279 marina du Chateau de Brest (Chateau, Atlantic) however, NIS were negatively impacted by warming,  
 280 just as were natives as shown by the constant proportional abundance. Yet, *Cryptosula pallasiana*,  
 281 native from the Mediterranean but introduced in the Atlantic, has been identified as an indicator  
 282 species of the heated treatment in the Chateau marina. No data on the thermal tolerance of this  
 283 species is available in the literature. However, it seems reasonable to assume that this tolerance should  
 284 be above the temperatures observed in the Atlantic marina due to its origin (Mediterranean Sea). In  
 285 its introduced habitat, it has been shown to almost double its growth rate at 18°C compared to 4°C  
 286 (Amui-Vedel et al. 2007), which may explain our observations. While anecdotal, this might indicate  
 287 that this species could have profited from our simulated warming.

288 The higher temperature did however not only influence the settlement dynamics on the panels, but  
289 also had an impact on the metabolism of our model species. In Carry we were unfortunately unable to  
290 sample *B. neritina* since too few individuals were present (mainly in the control). In the Chateau marina  
291 however, a slight difference between the metabolome of the heated and the control treatment is  
292 visible in the PCA and MVA test.

293 A total of 12 molecules had significant fold changes ( $FC > 1.5$ ) between the treatments. Most notably  
294 two phospholipids (Glycerophosphoethanolamine, Lyso-PE (22:6) and Glycerophosphocholine, Lyso-  
295 PC (22:6)) and a Bromo-Oxo-Quinolinecarboxylic Acid (BQA), were identified as significantly affected  
296 molecules. We were able to confirm the BQA structure and to match it with a commercially available  
297 standard for Caelestine A, a molecule described in the ascidian *Aplidium caelestis* (Yin et al. 2010). QA  
298 derivatives (especially brominated ones) have rarely been identified in nature and their exact function  
299 is thus difficult to establish (Yin et al. 2010). Caelestines have been shown to have minor cyto- and  
300 bacterio-toxicity at high concentrations (Yin et al. 2010). Quinolinecarboxylic acids (QA) seem to inhibit  
301 the activity of one of the most common protein kinases, Protein Kinase CK2 (Syniugin et al. 2016),  
302 which has numerous functions including inhibiting apoptosis and whose activity seems to increase with  
303 cellular stress (Manni et al. 2012; Piazza et al. 2012). Considering the very high overexpression of  
304 Caelestine A in the heated treatment and the implication of QAs with the inhibition of CK2, this  
305 molecule might be linked to the stress response of *Bugula neritina* to heat stress. While yet speculative  
306 the implication of Caelestine A in heat stress response should be further investigated in future  
307 experiments with *B. neritina* and other species like *A. caelestis*.

308 Two phospholipids varied between the control and the heated treatment (Lyso-PC (22:6) and Lyso-PE  
309 (22:6)). Phospholipids play a major role in cell membrane fluidity, which changes as a response to  
310 ambient temperature (Pruitt 1990; Cuculescu et al. 1995; Sung et al. 2003; Chen et al. 2016). The type  
311 of lipids used as well as the number of unsaturation is a key component in this response (Pruitt 1990;  
312 Cuculescu et al. 1995). It is interesting to note that in our case, both phospholipids have the same  
313 adducts. Lyso-PC (22:6) might results directly from the conversion of Lyso-PE (22:6) trough successive  
314 methylation by the phosphatidylethanolamine N-methyltransferase (Gibellini and Smith 2010),  
315 indicating that this conversion is associated to the increased heat. In the marine seaweed *Pyropia sp.*  
316 long term warming and short term heat shocks modified Lyso-PC and Lyso-PE concentrations generally  
317 increasing the first and decreasing the latter (Chen et al. 2016; Song et al. 2018), which corresponds to  
318 our observed results. Furthermore, PC and PE of different length including PC (22:6) and PE (22:6) have  
319 been shown to vary in the crabs *Carcinus maenas* and *Cancer pagurus* in respect to temperature  
320 (Cuculescu et al. 1995). The same Lyso-PE has also been previously described in *B. neritina*, varying  
321 according to environmental conditions in the Chateau marina (Gauff et al. in prep a). It may constitute

322 a marker of good environmental conditions as Lyso-PE may play a role in gametogenesis in certain  
323 marine organisms (Ivanisevic et al. 2011).

324 It is interesting to note that despite apparent similarities of the effect of +3°C warming in both marinas  
325 (Community differences, lower biomass, more empty space), the effect of warming on NIS prevalence  
326 was opposite, including *B. neritina*: in the Chateau marina the higher temperature led to lower  
327 abundance of NIS indicating that the generated heat stress may cause a disadvantage. This is further  
328 supported by the metabolomic differences between heated and control treatment in the Chateau  
329 marina, potentially indicating heat stress in *B. neritina*. Yet, in Carry-le-Rouet NIS, including *B. neritina*,  
330 had an increased abundance in the heated treatment. This last result is more in adequation with  
331 anterior results from the literature, with opportunistic species and NIS having higher biomass in warm  
332 treatments (Smale et al. 2011, 2017; Ashton et al. 2017). Moreover, an increased growth of *B. neritina*  
333 under higher temperatures has also been reported (Lord 2017) and numerous NIS, including *B.*  
334 *neritina*, have been shown to have high thermal tolerance (Hitoshi and Kazutsugu 1984; Zerebecki and  
335 Sorte 2011; Lejeusne et al. 2014; Lord 2017; Kenworthy et al. 2018a). However, strong variation (up to  
336 2°C) in temperature tolerance among geographically separated populations of several fouling species,  
337 including *B. neritina*, has been reported (Sorte et al. 2011; Zerebecki and Sorte 2011). This may be due  
338 to adaptation and/or acclimation of populations to their local climate and its variability (Sorte et al.  
339 2011). Tolerance to temperature-related stress may be acquired through numerous cellular pathways  
340 (Sung et al. 2003). Such tolerance can be increased in an invasive species by the exposure to stress  
341 (Clutton et al. 2021) by modifying epigenetics (Ardura et al. 2018) or be modulated by maternal effects  
342 (Burgess and Marshall 2011). High temperature heatwaves in a marina close to Carry-le-Rouet have  
343 been shown to lead to summer mass mortality for numerous species including *B. neritina* (Gauff et al.  
344 in prep b). It may be possible that the regular exposure of Mediterranean sessile communities to high  
345 temperatures and heat waves (Garrabou et al. 2009; Lejeusne et al. 2010) increases the thermal  
346 tolerance of present NIS, thus allowing their spread in warmer water. Conversely, the water  
347 temperature in the vicinity of the Chateau marina is more constant due to rarer heatwaves and higher  
348 water mixing due to higher tides and wave action (no stratification). This may explain why the  
349 simulated warming strongly impacted the present NIS which would not be accustomed to such  
350 disturbance.

351 The present results indicate that in the short term, climate change related ocean surface warming  
352 might not have the same effect on the prevalence of non-indigenous species everywhere. Certain  
353 locations, where regular heat events are already experienced, might have a higher risk at favoring them  
354 compared to cooler and more stable climates. This might be attributed to heat resistant individuals  
355 being favored by the intense selection exerted by heat waves. The impact of climate change might thus

356 also depend on how global warming modifies cooler temperate climates and if this increases extreme  
357 weather events like heat waves. It is crucial that further studies investigate *in situ* how heat waves and  
358 global warming influence marine urban communities and impact of NIS within. These studies might  
359 also integrate the conjoint effect of ocean acidification due to increased CO<sub>2</sub> concentrations and  
360 temperature as they both are likely to highly influence, which species profit from climate change.

361

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613 The authors have no relevant financial or non-financial interests to disclose.

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