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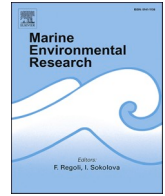
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Fouling community shows high resistance and metabolic resilience towards experimental high intensity heatwave

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ABSTRACT

Climate change is predicted to increase the prevalence of marine heatwaves with an increase in heatwave frequency and intensity. While some studies have shown the effect of marine heatwaves in warm temperate climates and the effect of overall higher temperature in warm and cold temperate climates, it is yet not entirely understood how heatwaves impact marine urban communities in cold temperate climates. As thermal resistance might be related to selective pressures and acclimation, it seems reasonable to assume that they may have a strong impact on local fauna and flora. In the present study, we simulated an *in situ* high amplitude heatwave and observed the community structure and the metabolism of *Bugula neritina* at two time-steps after the heatwave and compared them to control communities and individuals. Contrary to our expectations, the community structure remained vastly unaffected, as did the total metabolome of *B. neritina*. This shows that the community was able to resist the disturbance of the heatwave. *Bugula neritina* additionally showed a certain metabolic resilience as the already minor differences in the metabolome between control and the heatwave treatment diminished even further between the tested time steps.

1. Introduction

Climate change is well underway. Despite certain political efforts to combat it (UN, 2015; IPCC, 2021), the aim of limiting global warming to +1.5 °C targeted by the Paris agreement seems decreasingly likely as the +1 °C mark has already been passed (IPCC, 2021). In association with atmospheric warming, sea surface temperature is also projected to increase, with estimates ranging from +0.7 to +3 °C above preindustrial levels (IPCC, 2019). However, climate change cannot simply be reduced to overall warmer temperatures as it also modifies weather patterns and favors extreme weather events such as heatwaves (Meehl et al., 2000; Holl, 2009; Stott, 2016). Like global warming affecting the sea surface temperature, heatwaves can also impact the marine realm. This has led to an increase in the frequency and intensity of marine heatwaves in recent decades (Smith et al., 2023).

Past marine heatwaves have been shown to have drastic impacts on local communities (reviewed in Smale et al., 2019; Smith et al., 2023), often associated with the die-off of certain key species (Garrabou et al.,

2009; Lejeusne et al., 2010; Sorte et al., 2010a; Strydom et al., 2020). These effects are observable in natural environments, but also in urbanized areas (Smale et al., 2015; Gauff et al., 2023) where the heat is often trapped and amplified (Arnfield, 2003; Chapman et al., 2017). Past research on the subject of marine heatwaves and their effect on marine communities has mostly laid in assessing impacts after their occurrence (Garrabou et al., 2009; Lonhart et al., 2019; Strydom et al., 2020; Gómez-Gras et al., 2021; Gauff et al., 2023). However, due to the unpredictability of heatwaves, such studies often have issues in clearly linking the heatwave to the observed perturbation (Garrabou et al., 2009; Gauff et al., 2023). As a solution, simulated heatwaves are increasingly used in mesocosms or aquaria (Sorte et al., 2010a; Pansch et al., 2018; Castro et al., 2021) or *in situ* (Smale et al., 2015) to study their effects.

In past studies, we noticed differential responses to *in situ* simulated climate change of +3 °C in a Mediterranean and Atlantic marina (Gauff et al., 2022b). Mediterranean marine communities experience regular high temperature heat events (Garrabou et al., 2009; Lejeusne et al.,

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2010; Gauff et al., 2023) contrasting from more stable North Atlantic climate where heatwaves have lower intensity, which has been put forward as a potential explanation for this observation (Gauff et al., 2022b). Indeed, different populations of the same species may have differential thermal tolerance (Sorte et al., 2011; Zerebecki and Sorte, 2011) which may be linked to their adaptation/acclimation to the local climate (Sorte et al., 2011). Exposure to thermal stress has been shown on multiple occasions to increase thermal tolerance of individual species (Sung et al., 2003; Burgess and Marshall, 2011; Clutton et al., 2021). Due to the unpredictability of marine heatwaves in North Atlantic regions and due to difficulties of deploying *in situ* experimentations, this remains however difficult to prove.

In the present study, we use an *in situ* temperature modulating structure to simulate an intense heatwave in a North Atlantic marina to observe its effect on community structure and the metabolism of one of its key species, *Bugula neritina* (Linnaeus, 1758). As exposure to regular heat stress seems to increase thermal resistance (see above), we expected the community from a relatively stable climate to be highly susceptible to this heatwave. We expected a drastic difference in community structure between the heatwave and control treatment, with a major mortality of its species in the heatwave treatment. We also expected a strong impact of the heatwave on the metabolism of *B. neritina* (here illustrated by its metabolome) with consequences lasting beyond the immediate time scale.

2. Methods

2.1. Experimental setup

The experiment was conducted in the Chateau Marina (48° 22' 43.4" N; 4° 29' 22.1" W) in Brest, France. An *in situ* temperature regulating structure, consisting in $n = 10$ settlement plates was deployed at the outermost pontoon of the marina to avoid the effect of strong pollution in other areas (Kenworthy et al., 2018b; Gauff et al., 2022a; Rondeau et al., 2022). The settlement plates (20 cm × 20 cm) were made of polypropylene (Correx) and roughened with sandpaper to be more bioreceptive. They were all incased in transparent acrylic cylinders, including controls (control, $n = 5$), to avoid biases linked to the water circulation. Four 0.5 cm² passthroughs at the top of the cylinders increased connectivity with the outside water to avoid excessive stratification inside the cylinders. Some of cylinders ($n = 5$) were equipped with 200 W aquaria heating elements spaced around 20 cm from the panels (Gauff et al., 2022b), controls had a plastic tube instead. This attribution was done randomly to reduce biases from their spatial distribution. Each cylinder was spaced 15 cm from others to reduce heat exchange. We let benthic colonization of the structures proceed for 4 months between May and August which allows for ample colonization (Gauff et al., 2022a; Rondeau et al., 2022). After this initial colonization phase, the structures were cleaned to avoid fouling in undesired areas such as the acrylic cylinders and the heating elements and then the heatwave program was initiated (T0, Sept. 1st). During this time, a built-in computer (Rasberry Pi3+) coupled to DS18B20 cable thermometers installed at 0 cm, 5 cm, 10 cm away from each settlement plate, as well as outside of the cylinders regulated and monitored the temperature. Nominal temperatures were obtained when the thermometers at 0 cm and 5 cm from the plates reached outside temperature plus target increase. The heatwave simulation lasted for 8 days following the daily sequence +0.5 °C, +1.5 °C, +3.5 °C, +5 °C, +5 °C, +3.5 °C, +1.5 °C, +0.5 °C (Fig. 1). A heatwave can be defined by an event with temperatures exceeding the 90th percentile of historic data for more than 5 days (Hobday et al., 2016). We consulted temperature data from

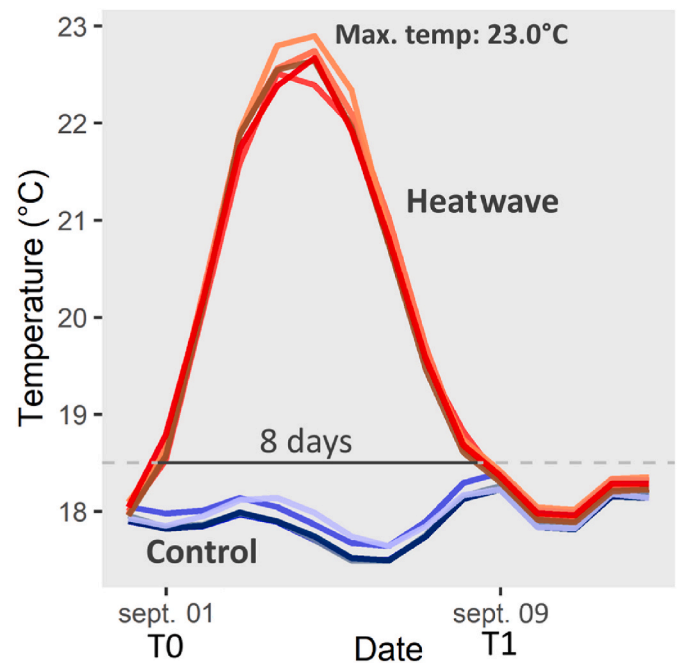


Fig. 1. Measured temperature curves of the *in situ* simulated heatwave ($n = 5$, red colors) and the control treatment ($n = 5$, blue colors). The dashed line represents the heatwave threshold for the given period calculated from the 90th percentile of historical data (Hobday et al., 2016). T2 is outside of the frame (sept. 14).

SOMLIT (Service d'Observation en Milieu Littoral; <http://www.somlit.fr>; consulted the Feb. 10, 2023) surveillance station at Brest: Portzic from 0–1 m for August and September for the years 2006 (beginning of time series) to 2022 as reference. To account for initial differences of temperatures between inside and outside of the marina, we re-calibrated the 90th percentile of this data (17.82 °C) by adding the measured difference in water temperature between the mean value of our measurements in the marina (17.94 °C from 31/08 to 14/09) and the mean water temperature in the SOMLIT data for the same period (17.49 °C from 31/08 to 14/09; Sup. Table 1). This adjusted 90th percentile threshold for defining a heatwave was thus fixed at 18.27 °C (rounded to 18.5 °C for convenience). Plotting our temperature measures in the heated and control units showed that the setup did indeed successfully pass the threshold for the whole duration of the program with a thermal maximum of 4.5 °C above the threshold.

2.2. Community analysis

Five days after the end of the simulated heatwave, all settlement plates were detached and brought into the laboratory for analysis. Communities were kept alive in aquaria and analyzed within 36 h. A 100-point grid was superposed over an area of 15 cm × 15 cm on the panels allowing for an accurate estimation of the contribution of species with more than 5% cover with a confidence interval of 95% (Taormina et al., 2020). All strata of the community were considered, even if species overlapped. Species that were present on the plates, but not on an intersection were accounted for with 1% cover. After the community analysis, the entire communities were dried in an oven (60 °C) for one week and burned at 520 °C for 6 h to obtain the Ash Free Dry Mass (AFDM).

Table 1

Highlighted features of the metabolome with a significant fold-change (FC) between the heatwave treatment vs. control treatments for *Bugula neritina*. Notes provide information about annotation, for example regarding the detection of the same molecule in MS1⁺ and MS1⁻, or ‘*’ the formation of a formic acid adduct that loses first C₂H₄O₂ (HCOO + CH₃ from trimethyl ammonium) during fragmentation (Zhang and Reid, 2006). Annotation confidence levels (Schymanski et al., 2014) are indicated: 4 Unequivocal molecular formula, 3 Tentative candidate (structure), 2 b Probable structure by diagnostic evidence. Rt = Retention time (in s), Var.: Under-expressed (↘) or over-expressed features (↗), FC = Fold-change, Conf = annotation confidence levels, Frag.: fragment. For more detail on annotation see Sup. Table 3.

Features	m/z	Rt	Var.	FC	p value	Molecular Formula	Note	Annotation	Conf
T1									
NEGM162T493	162.0231	493	↘	0.3	0.034	C ₁₉ H ₂₇ NO ₅ S	In-source frag.	C ₁₉ H ₂₇ NO ₅ S	4
NEGM498T594	498.2630	594	↗	2.5	0.029	C ₂₅ H ₄₂ NO ₇ P	–	Lyso-PE (20:5)	2 b
POSM500T595	500.2774	595	↗	2.2	0.019	C ₂₅ H ₄₂ NO ₇ P	same as previous		
NEGM586T598	586.3154	598	↗	2.5	0.016	C ₂₈ H ₄₈ NO ₇ P	[M + HCOO] ⁺ *	Lyso-PC(20:5)	2 b
POSM542T598	542.3244	598	↗	2.1	0.045	C ₂₈ H ₄₈ NO ₇ P	same as previous		
NEGM612T623	612.3303	623	↗	2.1	0.016	C ₃₀ H ₅₀ NO ₇ P	[M + HCOO] ⁺ *	Lyso-PC(22:6)	2 b
POSM568T623	568.3406	623	↗	2.3	0.018	C ₃₀ H ₅₀ NO ₇ P	same as previous		
NEGM380T644	380.2479	644	↘	0.6	0.022	C ₁₈ H ₃₉ NO ₅ S	–	C ₁₈ H ₃₉ NO ₅ S	4
T2									
POSM237T464	237.2044	464	↗	4.7	0.006	C ₁₅ H ₂₅ NO	isotope	C ₁₅ H ₂₅ NO	4
NEGM448T558	448.3047	558	↗	2.0	0.049	C ₂₉ H ₄₀ N ₂ O ₂	isotope	C ₂₉ H ₄₀ N ₂ O ₂	4

2.3. Metabolome extraction

The bryozoan *Bugula neritina* was sampled for metabolome analyses. This species has already been targeted for metabolome analyses in marinas in the past, including in experiments focusing on temperature (Gauff et al. 2022a, 2022b). Five samples (n = 5 replicates) of high-quality colonies (no epibionts, clean, alive) were taken just before initiation of the heatwave program (T0), as well as for both treatments at the end of the heatwave program (T1; n = 5 replicates per treatment) and 5 days after the end of the heatwave program (T2; n see below). This last sampling allowed us to observe the metabolic resilience of the sampled species. Sampled colonies were stored at –80 °C and dry-frozen and ground to dust. Due to mortality of *B. neritina* between T1 and T2, samples of T2 only reached 5 mg per sample as opposed to 15 mg for T1. This unfortunately hinders simultaneous injections and direct comparisons between T1 and T2, but we preferred to not align T1 samples on T2 as this could have increased methodological risks and could have decreased the quality of the analysis. Biomass in T0 samples was sufficient to create 5 mg as well as 15 mg subsamples allowing to compare T1 and T2 with T0. For T2, one sample needed to be pooled from two others to reach this mass (T2; n = 5 for Control, n = 4 for Heatwave).

Samples were extracted thrice with 1 mL MeOH/DCM (1:1 v:v) and 3 min of ultrasonication. Supernatants were filtered on PTFE syringe filters (13 mm, 0.22 µm, Restek®) and transferred into vials with 15 mg of silica powder (C-18 Polygoprep® 60–50, Macherey-Nagel®) for the 15 mg of biomass samples (T0, T1). For 5 mg biomass samples (T0, T2), the filtered supernatants were directly transferred into vials. All samples were dried under the hood for 24 h, then in a vacuum concentrator for MeOH removal (SpeedVac®, SPD111V, Thermo Scientific® Savant). While 5 mg samples (T0, T2) were directly resuspended in 500 µL of MeOH for analysis, 15 mg samples (T0, T1) were submitted to additional cleaning to remove salts. The dried extracts on silica were deposited on SPE C-18 cartridges (Strata® C18-E 55 µm, 70 Å, 500 mg/6 mL, Phenomenex®) that were previously rinsed with 2 x 5 mL MeOH, dried and conditioned with 2 x 5 mL of H₂O. The extracts were flushed with 3 x 5 mL of H₂O and then extracted with 5 mL of MeOH. The extracts were dried and resuspended in 500 µL of MeOH for analysis. Blank samples going through the entire extraction process without initial biological material were made for both workflows. Additionally, pooled samples were made for each workflow by combining 30 µL of each sample.

2.4. Metabolome analysis

Analyses were performed using an UHPLC (3000 RS equipped with an automatic injector, a regulated-temperature column oven and a Photo-Diode Array detector, Thermo®) coupled to a High Resolution Mass Spectrometer (Impact II equipped with an ESI source, Bruker Daltonics®). Metabolites were separated on an Acclaim™ RSLC 120 C18 column (150 mm × 2.1 mm, 2.2 µm, ThermoScientific®) with an elution rate of 0.5 mL min⁻¹ at 40 °C. Elution solvents were composed of water (LC/MS grade, Carlo Erba®) + 0.1% formic acid (A) and acetonitrile (LC/MS grade, Carlo Erba®) with the same adjuvant (B). The chromatographic program followed the steps: 5% (A) during 2 min; linear increase up to 100% (B) during 8 min; maintaining 100% (B) during 4 min. Initial conditions were then rapidly reached (0.01 min) and maintained for 2.99 min for a total sample analysis of 17 min. Volume injections were tested with pooled samples at 1, 2, 5, 10 and 20 µL for the 5 mg extracts and 1, 2, 5 and 10 µL for the 15 mg extracts in positive and negative modes before the analysis of metabolomic samples. Finally, a volume of 5 µL (for 5 and 15 mg extracts) was injected for MS1⁺ acquisitions, and 10 µL (for both extracts) for MS1⁻ acquisitions. A volume of 10 µL of both extracts was also injected for MS2 acquisitions in both modes.

MS parameters were set as follows for positive and negative modes: 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 in MS1 (4 Hz at 20–40 eV collision energy for MS2). The spectrometer was calibrated with a formate:acetate solution in basic media (Bruker) forming clusters on the mass range before analysis. The same solution is injected before each sample allowing an automatic re-calibration of detected masses (m/z precision <2 ppm) and netCDF exportation with a Bruker script in Bruker Compass DataAnalysis (ver. 4.3). A separate pooled sample for 5 and 15 mg extracts was injected at regular intervals in each sequence (every 7–8 samples) to correct for possible mass spectrometer drift during the sequence and to remove variable intensity ions from matrix. Blank samples were also injected to filter for ions linked to the solvent and the extraction process.

2.5. Metabolome matrix

Exported netCDF files were processed using the R package ‘XCMS’

(version 3.12; Smith et al., 2006) in separate sequences. Automatic peak picking for feature detection was followed by a retention time correction (obiwarp method) and by feature grouping in fixed time frames (5 s). Peak filling step integrated portions with initially absent peaks. Afterwards, the report and data matrix were then generated in a Microsoft Excel format. This table was submitted to three in-house filtering steps on R that permit to remove 1/ions from blanks compared to pooled samples, 2/variable intensity ions in pooled samples, 3/auto-correlated ions to remove isotopes (>80%).

2.6. Computational and statistical analysis

The community matrix (Sup. Table 2) has been analyzed in R (version 4.0.3; R Core Team, 2020) using the 'vegan' package (version 2.4–6; Oksanen et al., 2018). The Shannon and Simpson diversity indices as well as the Pielou Evenness and the species richness were calculated for each sample. Significant differences between control and the heatwave treatment were assessed with Mann-Whitney-Wilcoxon tests (hereafter M-W-W test). Differences in AFDM were assessed in the same manner. The community matrix was transformed into a Bray-Curtis dissimilarity matrix. This was done without pre-treatment transformations as this study focuses more on brought differences in community structure between treatments. Indicator species for each treatment were identified with a multipattern analysis from the 'indicpecies' package (version 1.7.14; De Cáceres and Legendre, 2009; De Cáceres, 2013). The dissimilarity matrix was then projected into a Principal Coordinate Analysis (PCoA). Homogeneity of dispersions has been verified via the 'betadisper' test. A PERMANOVA (10^6 permutations) tested for a difference between control communities and those submitted to the experimental heatwave.

The metabolome matrices of each injection mode ($MS1^+$, $MS1^-$) were fused together for 15 mg extracts (comparing T0 to T1) and for 5 mg extracts (comparing T0 to T2). The two resulting metabolome matrices (<5000 features) was analyzed in 'MetaboAnalyst' (version 5.0; Xia et al., 2009). Data were Log-transformed and auto-scaled and PCAs were realized, first including T0 and then excluding it, to focus on differences between the heated treatment and control. Significant differences in the metabolome were identified in R with a PPLS-DA driven permutation Model Validation Analysis (MVA; 10^3 permutations, 6 components) and

an associated pairwise test from the 'RVAideMemoire' package (version 0.9–79; Hervé, 2021). A Volcano plot helped to identify features that had significant ($p < 0.05$) fold-changes above 1.5. These features were targeted for annotation using fragmentation mass spectra ($MS2^+$, $MS2^-$). Bruker DataAnalysis has been used to study the fragments of each precursor of interest.

3. Results

3.1. Community

A total of 22 species were identified during the study, 6 of which were Non-Indigenous Species (NIS). Species richness per panel ranged from 11 to 16 species, but no significant differences in richness were identified between the heatwave treatment and control (M-W-W test, $W = 7$, $p = 0.28$). The Shannon diversity index, Simpson diversity index, as well as Pielou evenness had higher values in the heatwave treatment compared to the control treatment (respectively mean: $H' 2.57$ vs. 2.16 ; $\lambda 0.72$ vs. 0.6 ; and $J' 0.67$ vs. 0.58) but differences were not significant (M-W-W test, $W = 3$, $p = 0.056$). AFDM was constant between treatments (Fig. 2A; M-W-W test, $W = 8$, $p = 0.42$).

The most abundant space occupiers were *Bugula neritina* (39% on average among all plates), *Ciona intestinalis* (10%), *Clytia hemisphaerica* (6.7%). None of these species had a significantly different surface cover between the treatments, although *B. neritina* was close to significance (Fig. 2B; M-W-W test, $W = 21$, $p = 0.094$). No species was identified as indicator species of any treatment by the multipattern analysis. While the PCoA had a high percentage of explained variability (76.8%), and PCo1 (54.6%) seemed to be associated to the treatment (Control -PCo1 and Heated + PCo1), communities between the heatwave treatment and the control had a notable overlap and were not significantly different (Fig. 2C; PERMANOVA, $R^2 = 0.23$, $p = 0.079$).

3.2. Metabolome

The two PCAs including T0 and the PPLS-DA driven pairwise MVA tests both showed that T0 metabolomes highly differentiated from those of T1, control and heated (Fig. 3A; PC1 58.6%; Pairwise MVA, $p < 0.014$) as well as from those of T2, control and heated (Fig. 3C; PC1

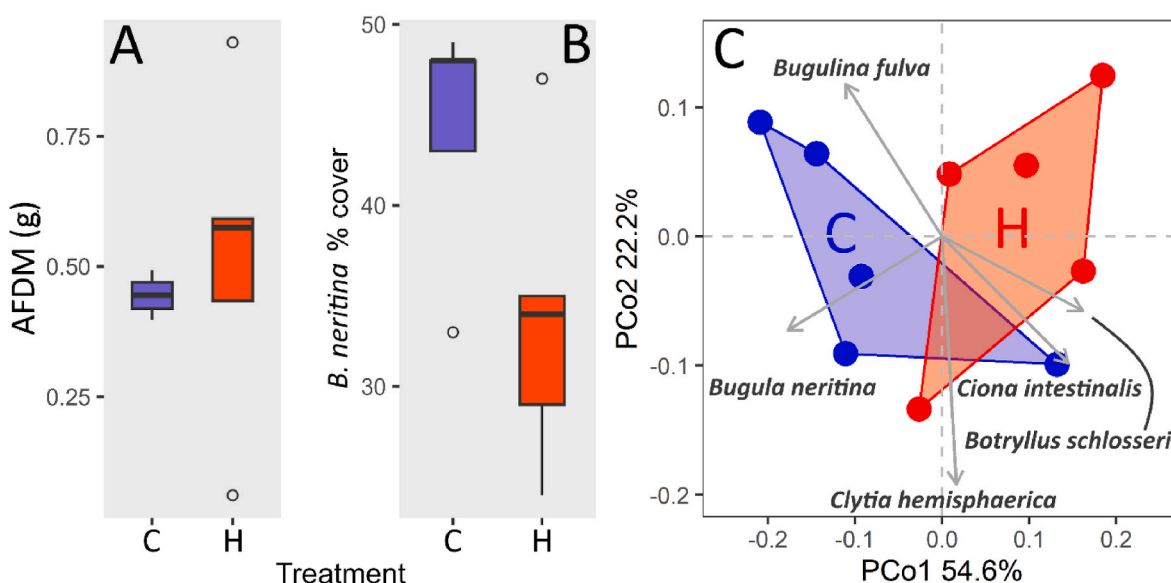


Fig. 2. A) Boxplot of the Ash Free Dry Mass (AFDM in g = biomass) of the total community in control and the heatwave treatment. B) Boxplot of the Percent cover of *Bugula neritina* in control and the heatwave treatment. C) PCoA of the community structure of control (C, Blue) and communities submitted to the heatwave treatment (H, Red). Vector overlay indicates species with a positive correlation to the projection ($R^2 > 0.3$; $p < 0.05$). No significant differences between C and H for any of these results.

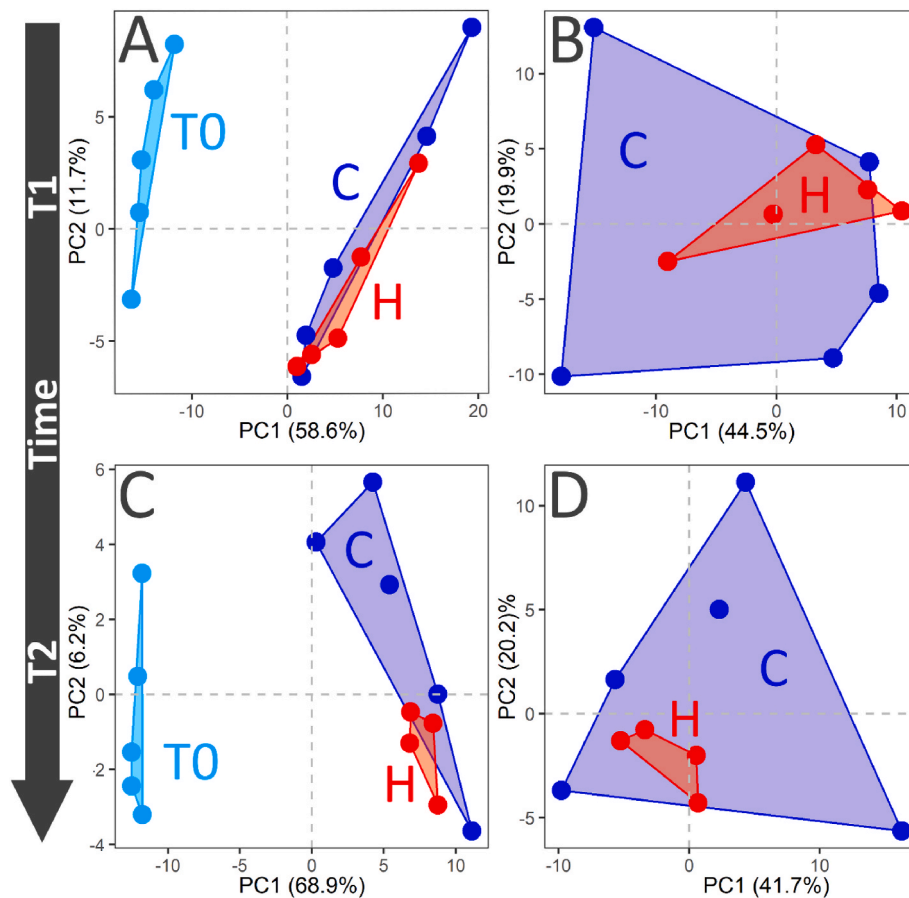


Fig. 3. PCA of the metabolome of *Bugula neritina* A) for control at T1 (Dark Blue; C) vs. the heatwave treatment at T1 (Red; H) compared to the metabolome at T0 (Light Blue), B) for control vs. the heatwave treatment at T1, C) for control at T2 vs. the heatwave treatment at T2 compared to the metabolome at T0, and D) for control vs. the heatwave treatment at T2.

68.9%; Pairwise MVA, $p < 0.049$). T1 and T2 control metabolomes were thus always distinct from T0 which did not permit the direct comparison of T1 and T2. When focusing only on the control and heated treatments at T1, PC1 and PC2 explained over 64% of the observed variability (Fig. 3B), with an additional 10% on PC3 (Sup Fig. 1B), which allowed to somewhat discriminate the treatments. At T2, PC1 and PC2 explained over 61% of variability (Fig. 3D) with 10% added by PC3 (Sup. Fig. 1D). However, at T2 the overlap between control and heated was high, even when considering PC3. At both time steps, no significant differences were found in the overall metabolome between control and heated (MVA test, Classification Error Rate = 0.44, $p > 0.49$).

Detailing individual features varying between treatments, 12 of them at T1 had a significantly different fold-change (FC) of at least 1.5 in the heatwave treatment compared to control (9 over- and 3 under-expressed; Fig. 4A). At T2, only 4 such features were highlighted (3 over- and 1 under-expressed; Fig. 4B). A consequent part of the features identified in T1 are however redundant, as they correspond to multiple detections of a molecule in both modes (see Table 1).

4. Discussion

Marine heatwaves are projected to increase in frequency and intensity due to climate change (Frölicher et al., 2018). However, as up now it is difficult to plan them in advance and most studies opportunistically assess their impact during or after occurrence and proper controls often lack (ex: Gauff et al., 2023). Studying their effect is thus challenging. The aim of the present study was to use an *in situ* device to simulate a heatwave and to observe the effect on the fouling community of a marina and the metabolism of one of its key species. The

experimental setup effectively simulated, as expected, a high intensity heatwave lasting 8 days over the 90th percentile of historic data (Hobday et al., 2016), surpassing this threshold by over 4 °C in the heated treatment (reaching a max. temperature of 23 °C). Contrary to our predictions however, the heatwave had only a minimal impact on community with no significant differences on structure and cover of any species and no significant impact on the overall metabolome of *Bugula neritina*. The marina community has thus demonstrated a high resistance to the applied disturbance.

The community structure was remarkably similar between the two treatments. While some variation in biodiversity indices like the Simpson index, Shannon diversity index or the Pielou Evenness could be observed, none of the here tested metrics showed significant differences, although they were sometimes close. Higher biodiversity in the heatwave treatment is consistent with the Intermediate Disturbance Hypothesis (Connell, 1978; Hughes, 2010). However, given the overall low impact of the heatwave on other metrics, strong conclusions in this regard remain objectionable. The total community structure was close to be significantly different (PERMANOVA, $R^2 = 0.23$, $p = 0.079$) likely due to almost significant differences in the cover of *B. neritina* (M-W-W test, $W = 21$, $p = 0.094$), the dominant species in our study. These results, however, remained worlds apart from the expected disruption by the heatwave. Rising temperature has been shown to strongly impact communities (Lejeune et al., 2010; Smale et al., 2011, 2017; Sorte et al., 2011; Gauff et al., 2022b) and particularly heatwaves have been shown to have severe consequences on marine communities, their physiology, and their ecosystem services (Garrabou et al., 2009; Sorte et al., 2010a; Smale and Wernberg, 2012; Smale et al., 2017; Britton et al., 2020; Gauff et al., 2023). The impacts of high temperature events can last for tens of

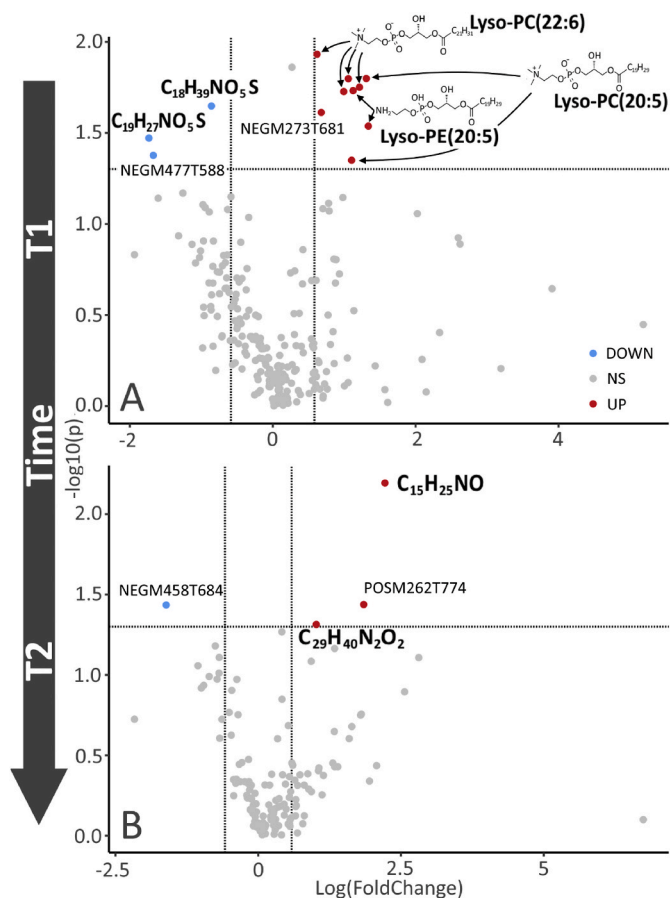


Fig. 4. Volcano plot showing features of the metabolome of *Bugula neritina* with a significant (t -test, $p < 0.05$) fold-change ($\text{FC} > 1.5$) in the heatwave treatment vs. control (indicated by colored dots on plot) for A) T1 and B) T2. Left (blue dots): under-expressed features compared to control. Right (red dots): over-expressed features compared to control. The x-intercepts correspond to -1.5 and 1.5 FC. The y-intercept corresponds to a p -value of 0.05 . Annotations from Table 1 indicated.

years after occurrence (Gómez-Gras et al., 2021). The here observed invariance of community structure exposes a clear case of community resistance, as the community resisted the disturbance without changing (Lake, 2013; Nimmo et al., 2015), in opposition to resilience, a community's capacity to return to a stable state after disturbance (Holling, 1973; Lake, 2013). The tested time scales here (days) do not allow for resilience to take place in the community structure (however a certain metabolic resilience in *B. neritina* can be noted; discussed below). Previously, a 50 day warming experiment in the same marina with the same *in situ* device, heating communities $+3$ °C above ambient temperatures, has shown a strong effect on recruiting community structure as well as the abundance and metabolism of key species like *B. neritina* (Gauff et al., 2022b). It is thus intriguing to note that, while long term heating significantly impacted community structure in the past study, it seemed much more resistant to the pulse disturbance exerted by the heatwave. A previous study simulating heatwaves of different intensities on marina communities also showed high resistance towards the disturbance (Smale et al., 2015).

No significant differences in the overall metabolome of *B. neritina* could be observed between the heatwave and control treatment in T1 or T2, despite some molecules significantly varying between the two treatments. More notable is the difference between T0 and both other time steps, illustrating that temporal variation, whatever its cause (environmental variation except temperature, biotic interaction, growth ...) is more important than the heatwave treatment. While resistance

and resilience in terms of metabolism are concepts generally used on the overall metabolism of communities (Uehlinger, 2000), they may also apply to metabolomes within one species like here. Thus, we might observe here a case of metabolic resistance of *B. neritina* to the disturbance, however we are here not able to exclude metabolic resilience, as it may be possible that *B. neritina* already returned to its undisturbed state at T1 (~ 4 days after peak heat) due to the relatively short turnover time of metabolomes. The presence of some resilience is indeed supported by the higher number of features which had a significantly different expression levels between the two treatments at T1 (12) than in T2 (4) suggesting a quick return to a normal state. However, it is not possible to exclude reduced detection power at T2 due to the lower sample biomass as we detected only 137 features in T2 as opposed to 240 in T1. Some of the significantly affected molecules were glycerophospholipids, which have been described as potential fitness markers in *B. neritina* (Gauff et al., 2022a). As an example, lyso-PE (20:5) and lyso-PC(20:5) only differ by the replacement of the ethanolamine head by a choline, while Lyso-PC(22:6) has a slightly longer carbon chain than lyso-PC(20:5). Phospholipids in general intervene in cell membrane fluidity, which has been shown to be highly dependent to temperature (Pruitt, 1990; Cuculescu et al., 1995; Sung et al., 2003; Chen et al., 2016), altering lipid types and insaturation numbers (Pruitt, 1990; Cuculescu et al., 1995). Many phosphocholines [including specifically lyso-PC(22:6)] and phosphoethanolamines have been shown to be highly affected by heat shocks or higher temperature in crustaceans and algae (Cuculescu et al., 1995; Chen et al., 2016; Song et al., 2018). Moreover, lyso-PC(22:6) has already been identified as a molecule potentially associated to heat stress in *Bugula neritina* (Gauff et al., 2022b). This might illustrate that a slight response of *B. neritina* to the heatwave was present, at least immediately after the treatment application.

Information regarding *B. neritina* heat resistance is very contrasting. On one hand it has been described as very tolerant to high temperatures up to 30 °C (Hitoshi and Kazutsugu, 1984; Lord, 2017a, 2017b), which could explain the low effect of our simulated heatwave. On the other hand, *B. neritina* has been observed to be sensitive to high intensity Mediterranean heatwaves (Gauff et al., 2023), which were however notably warmer at 28 °C maximum as opposed to our 23 °C. Furthermore, *B. neritina* has been shown to have a reduced cover and a modified metabolism, expressing molecules that can be linked to heat stress, in a long-term heating experiment ($+3$ °C above ambient temperature) in the same marina as here (Gauff et al., 2022b). Geographically separated populations of *B. neritina* may show differences in temperature tolerance (Sorte et al., 2011) which can be explained by local adaptation, which has been observed in this species on multiple occasion for different stressors (Piola and Johnston, 2006b; Sorte et al., 2011; Gauff et al., 2022a). It is thus intriguing not to observe high metabolic variations of this species towards the simulated heatwave as we would expect a strong effect of the heatwave due to the rarity of such events in the North-East Atlantic and thus, theoretically, low adaptation to such events. It is possible that the simulated heatwave here did just not surpass the thermal tolerance threshold of *B. neritina*.

We here observed an unexpected strong resistance of the whole marina community and *B. neritina* towards the simulated heatwave. In accordance with observations of local adaptation in marina communities and this species (Piola and Johnston, 2006b; Sorte et al., 2011; Gauff et al., 2022a) and the idea that more regular heat stress increases thermal tolerance (Sung et al., 2003; Burgess and Marshall, 2011; Clutton et al., 2021), we hypothesized a strong sensitivity towards the heat disturbance with a strong shift in community composition and high thermal stress for *B. neritina*. This was however not the case. This would mean that the heatwave here may have constituted a Type I disturbance not overcoming the communities' resistance, as opposed to Type II and Type III which involve community resilience (Sutherland, 1981; Boudouresque et al., 2009). Likewise, a previous experiment simulating heatwaves on marina communities in aquaria in the UK did find

strikingly similar results with no apparent change in community structure (Smale et al., 2015). The authors explained their observations with high thermal tolerance and resilience of adult sessile fauna, but also with the realistic scale of their simulated heatwave. Marina communities have a high persistence, even dominance of introduced species (Rondeau et al., 2022), which are described to have resistant characteristics (Lenz et al., 2011; Gröner et al., 2011). This resistance can be to pollutants (Piola and Johnston, 2006b, 2006a, Crooks et al., 2011, Osborne and Poynton, 2019, Gauff et al., 2022a), but is also often described for thermal stress (Sorte et al., 2010b; Kelley, 2014; Lejeune et al., 2014; Lord, 2017a; Kenworthy et al., 2018a). This could mean that the high contribution of introduced species to the marina communities (here around 60% of total cover; see Sup. Mat.), increases the resistance of the community to disturbance. Future experiments should simulate different intensities of heatwaves in marina communities with different levels of contribution of introduced species to test this hypothesis.

CRedit authorship contribution statement

Robin P.M. Gauff: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Stephane Greff:** Writing – review & editing, Validation, Formal analysis, Data curation. **Olivier Bohner:** Methodology, Investigation. **Stephane Loisel:** Software, Methodology, Investigation. **Christophe Lejeune:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Dominique Davoult:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2024.106813>.

Data availability

The data is included in the Supplementary material

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