

Consistency of aquatic enclosed experiments: The importance of scale and ecological complexity

Filipa Paiva^{1,2} | Dennis Brennecke^{3,4,5} | Christian Pansch⁶ | Elizabeta Briski¹ 

¹GEOMAR, Helmholtz-Zentrum für Ozeanforschung Kiel, Kiel, Germany

²MARE – Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Edifício Madeira Tecnopolo, Funchal, Madeira, Portugal

³Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, Foundation, Büsum, Germany

⁴Department of Biology, Marine Biological Research Centre, University of Southern Denmark, Kerteminde, Denmark

⁵Leibniz Institute for Science and Mathematics Education, Kiel, Germany

⁶Department of Environmental & Marine Biology, Åbo Akademi University, Turku, Finland

Correspondence

Elizabeta Briski, GEOMAR, Helmholtz-Zentrum für Ozeanforschung Kiel, Düsterbrookweg 20, 24105 Kiel, Germany.
Email: ebriski@geomar.de

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Abstract

Aim: Marine and freshwater ecosystems are increasingly threatened by human activities. For over a century, scientists have been testing many biological, chemical and physical questions to understand various ecosystems and their resilience to different stressors. While the majority of experiments were conducted at small-scale laboratory settings, lately large mesocosm experiments have become more and more common. Yet, it still remains unclear how the scale (i.e. space) and ecological complexity (i.e. community versus limited number of species) of experiments affect the results and to what extent different experimental types are comparable.

Innovation: Here, we conducted two types of experiments, run at different scale and ecological-complexity levels (i.e. outdoor large-scale community-level mesocosm versus indoor small-scale two-species laboratory experiment), to assess the effects of marine heatwaves on two gammarid species.

Main Conclusions: Our approach detected differences in abundance and relative population growth between the two experimental types for one out of the two tested species, but no difference in heatwave impacts on any of the species, independently of which experimental type was used. The larger space in the mesocosm, accompanied with inclusion of the community, benefited this species, demonstrating stronger performance in the mesocosm than in the laboratory experiment. Though, our study design cannot directly distinguish if scale or ecological complexity of the experiments, or both, caused the observed discrepancy in our findings. Furthermore, inconsistency in results among laboratory experiments complicates the extrapolations and generalization of the laboratory results. Yet, our findings indicate the importance of space, density-dependent effects, biotic interactions and complexity of natural environments in buffering, or boosting, the direct effects of environmental stress on organisms. Therefore, we urge the use of large-scale community-level mesocosm experiments instead of small-scale single-species laboratory ones whenever possible, and emphasize a necessity of great caution when interpreting the results of laboratory experiments.

KEYWORDS

gammarids, heatwaves, laboratory experiment, large-scale experiments, mesocosm, small-scale experiment

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1 | INTRODUCTION

The speed and extent of current changes in the Earth's climate and environments have reached unprecedented rates, being faster than any previously observed (IPBES, 2019; Stillmann, 2019). Over the last several decades, marine and freshwater ecosystems have been significantly affected by rising temperatures and other anthropogenic activities, such as increased nutrient flows, overexploitation and introduction of non-native species (Capinha et al., 2015; Chapman, 2017). Consequently, scientists have been using field observations and experiments, often in combination with mathematical modelling, to understand coastal ecosystems and their resilience to stress (Petersen et al., 2009). Through time, due to development in science and technology, new and more advanced approaches and methods have been designed and developed, including valuable improvements in experimental set-ups, data analyses and computation (Evans, 2012; Woodward et al., 2010).

While some ecological research can be successfully conducted by observational studies with specific support of mathematical modelling, others need manipulative experiments to prove or refute the tested hypotheses (Stewart et al., 2013; Widdicombe et al., 2010). There is no standard experimental design that fits all research questions, and the approaches used may differ not only in spatial scale, but also in ecological complexity. While spatial scale is defined by variables such as length, area and volume, ecological complexity is characterized by species diversity and levels of ecological organization (Petersen et al., 2009). In this context, research approaches can vary tremendously. On the one side, there are laboratory experiments which are usually conducted at small-scale and individual or single-species levels. These experiments often test many of the basic biological, chemical, and physical questions in a controlled environment, allowing for high replication (Widdicombe et al., 2010). However, they are often simpler in terms of complexity and exclude important ecological and biological components present under natural settings. As they create an artificial environment, their validity often raises concern among the scientific community (Carpenter, 1996; Widdicombe et al., 2010; Stewart et al., 2013). On the other side, large-scale mesocosm experimental infrastructure and approaches have been significantly improved in recent years, allowing replication of "near-realistic" scenarios including important variables occurring in the natural environments by using subsets of natural ecosystems (e.g. Dzialowski et al., 2014; Kraufvelin et al., 2006, 2010, 2020; Pansch & Hiebenthal, 2019; Wahl et al., 2015). By creating almost natural ecological and biological dynamics of ecosystems while the tested variables are manipulated and controlled, the results and conclusions deduced from mesocosm experiments are usually assumed to be more reliable and predictive than laboratory tests (Kraufvelin et al., 2006, 2010, 2020; Petersen et al., 1999; Widdicombe et al., 2010). However, it should be emphasized that though mesocosm experiments use a subsample of a natural ecosystem, they are still a simplification of nature, and this should be considered when drawing wider conclusions from the observed results and when forecasting future scenarios (Petersen et al., 2009; Widdicombe et al., 2010).

Extreme isolated events associated with global warming, namely heatwaves, have lately raised the awareness of the scientific community due to their increasing frequency worldwide in both marine and freshwater ecosystems (Holbrook et al., 2019; Huber et al., 2012; Oliver et al., 2018). Heatwaves are warm isolated events that last for five or more days at temperatures warmer than the 90th percentile based on a 30-year historical baseline period (Hobday et al., 2016). There are summer and winter heatwaves, with many of the former having devastating impacts on ecosystems, while some of the latter being even beneficial (Cavole et al., 2016; Hobday et al., 2016). In 2003, in the Northwestern Mediterranean region, one of the first documented impacts of a heatwave occurred, causing extensive mortality among numerous benthic communities (Garrahou et al., 2009). Since then, several studies have reported similar events worldwide, such as the Western Australia heatwave in 2011 (Pearce & Feng, 2013) and the Northwest Atlantic heatwave only one year later (Mills et al., 2013). Field observations have determined strong responses of marine and freshwater environments to summer heatwaves, such as toxic cyanobacteria blooms (Joehnk et al., 2008), mass coral bleaching (Hughes et al., 2017) and extensive mortalities of important commercial fish species (Caputi et al., 2016). As these isolated climatic events can negatively affect aquatic communities, and it is expected that they will increase in their severity and frequency (Cavole et al., 2016; Smale et al., 2019), it is of great importance to understand the responses and resilience of ecosystems to this climatic abnormality to be able to better protect coastal habitats (Frölicher et al., 2018; Sorte et al., 2010).

Although a great number of studies has been conducted to determine the responses of single-species or communities to global warming and other anthropogenic impacts using mesocosm and laboratory experiments (e.g. Casties et al., 2019; Madeira et al., 2018; Pansch et al., 2018; Wahl et al., 2020), it still remains unclear how the type of the experiment (i.e. scale and ecological complexity) affects the outcome and to what extent the two types of experiments are comparable. In this study, we conducted two experiments using different scale- and ecological-complexity levels: (a) an outdoor large-scale community-level mesocosm and (b) a small-scale two-species laboratory experiment, to assess the effects of heatwaves on two gammarid species from the Baltic Sea. To be able to compare the results of the two types of experiments, after three months of rearing animals in different set-ups, relative population growth was calculated for each species and for each experimental type. We tested the null hypotheses assuming no difference in population growth: (a) for any of the species between the two experimental types; (b) between the two species in each experimental type; and (c) among different treatments.

2 | METHODS

2.1 | Specimen collection

Two species from the superfamily Gammaroidea (i.e. *Gammarus locusta* and *G. salinus*) were collected in April and May 2015 for the mesocosm and in April and May 2016 for the laboratory experiment. *Gammarus*

locusta was collected in Falkenstein, Germany (54°40' N 10°20' E), while *G. salinus* was collected in Kiel, Germany (54°33' N 10°15' E), two sampling locations only a few km apart (<10). Specimens were transported in their ambient water to the laboratories at GEOMAR in Kiel, where each individual was morphologically identified according to Köhn and Gosselck (1989). Additional species for the mesocosm experiment were collected within the Kiel Fjord between 4 and 6 May 2015 (i.e. *Zostera marina*, *Fucus vesiculosus*, *Cerastoderma edule*, *Idotea balthica*, *Littorina littorea* and *Mytilus edulis*; for details see Pansch et al., 2018).

2.2 | Experimental set-up

To determine whether the type of the experiment affects the results of the experiment, we have conducted two experiments using different scale- and ecological-complexity levels: (a) an outdoor large-scale community-level mesocosm experiment—hereafter referred as mesocosm experiment; and (b) a small-scale two-species laboratory experiment—hereafter referred as laboratory experiment, to assess the effects of heatwaves on *G. locusta* and *G. salinus*. The mesocosm experiment was conducted from 6 May until 20 August 2015, and the laboratory experiment from 17 May until 2 September 2016. The experimental set-up of the mesocosm experiment consisted of six thermally insulated large mesocosms, placed outdoors, with each being divided into two independent experimental tanks of 1,500 L (Figure 1a, c). Water has been completely exchanged daily by a constant flow-through of 1,800 L per day of unfiltered seawater allowing for natural fluctuations in both abiotic and biotic conditions (Wahl et al., 2015). Only temperature was manipulated (see below; Figure 2). Forty-eight individuals of each *G. locusta* and *G. salinus* were added to each experimental tank, together with two main habitat-forming macrophytes of Western Baltic Sea: *Z. marina* and *F. vesiculosus*, and their most abundant associated fauna: *C. edule*, *I.*

balthica, *L. littorea* and *M. edulis* (for details see Pansch et al., 2018). The experimental set-up of the laboratory experiment consisted of six water baths (52 L each), with two experimental tanks (13.5 L each) set inside each bath (Figure 1b, d). Temperature of water in the experimental tanks was manipulated by regulating water temperature of the water baths, following the same pattern as in the mesocosm experiment (see below; Figure 2). Here, we acknowledge that this two-by-two block design may have some effect on our results, but it was necessary for temperature regulation. Water in the experimental tanks has been completely exchanged approximately every hour by a constant flow-through of filtered seawater (20 µm). Twenty individuals of each *G. locusta* and *G. salinus* were added to each experimental tank. We emphasize here that there was a large difference between the density of gammarids per volume of water introduced into the two experimental scales (i.e. the mesocosm and laboratory experiments), but this high density of individuals in the laboratory scale was necessary to secure enough healthy individuals of different sex to start the populations. To improvise the natural environment to a certain degree, sand, stones, empty mussel shells and artificial mesh were added to tanks, while animals were fed ad libitum with *F. vesiculosus* and a mixture of commercial food flakes for crustaceans (Tetra Mix). During the experiments, the experimental tanks were covered by lids and continuously aerated. Over the course of both experiments (i.e. the mesocosm and the laboratory experiments), there was limited or no contact with the animals. At the end of the experiments, individuals were collected by a sieve (mesh size of 335 µm), stored in ethanol and later morphologically identified.

2.3 | Heatwave treatments

The experimental design consisted of three treatments: (a) control; (b) one heatwave; and (c) three heatwaves. Each treatment consisted

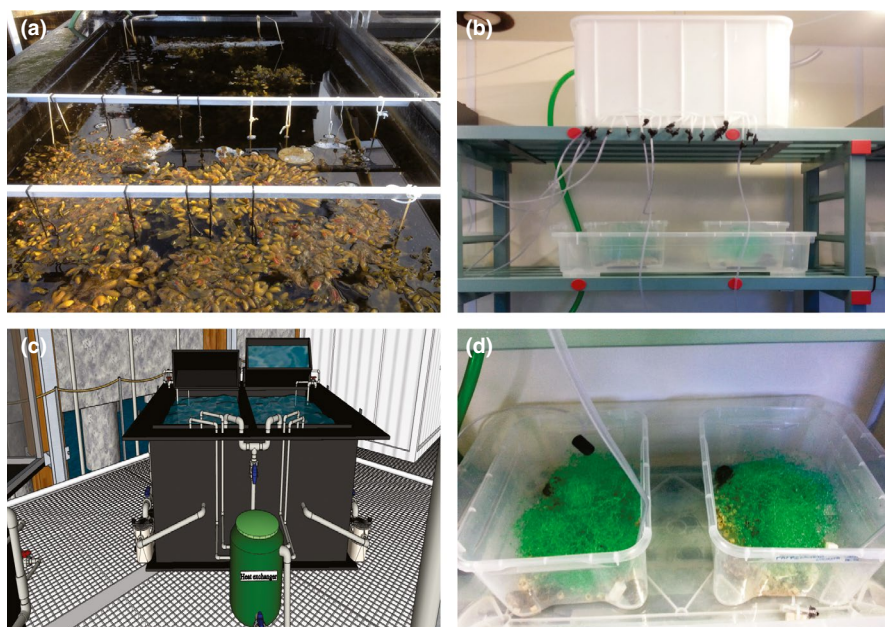
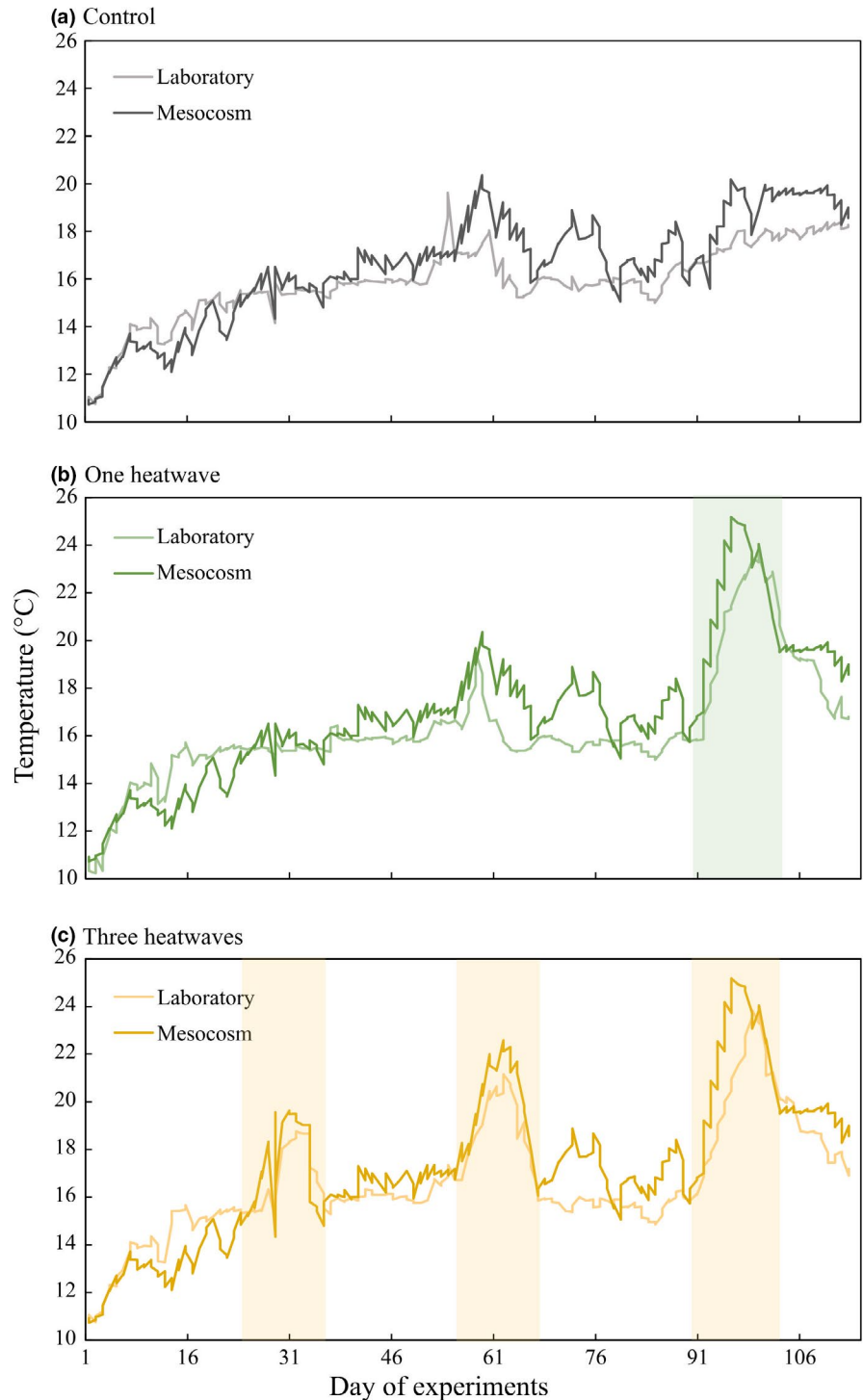


FIGURE 1 Overview of the two types of experimental set-up (a) mesocosm tank with tested community from above (see Pansch et al., 2018 and Wahl et al., 2015), (b) laboratory setting including the main water storage tank with water distribution hoses leading to the experimental tanks, (c) a scheme of the mesocosm tanks (Wahl et al., 2015), and (d) laboratory tanks with animals and artificial refugia structures from above

FIGURE 2 Implemented temperature profiles in mesocosm and laboratory experiments. Temperatures are provided from 6 May to 20 August 2015 for mesocosm and from 17 May to 2 September 2016 for laboratory experiment for (a) the control, (b) one heatwave and (c) three heatwave treatment. Heatwave periods are highlighted by green and yellow rectangles for the one and three heatwave treatment, respectively



of four replicates. The treatments were designed based on a statistical analysis of 15 years of daily mean temperature records from the experimental site. Using a generalized additive mixed model (GAMM), daily mean temperatures from May to September for the control treatment were designed (for details see Pansch et al., 2018; Figure 2). In the one heatwave treatment, the heatwave started on 90th day of the experiments, while in the three heatwave treatment, the heatwaves started on 25th, 56th and 90th day, respectively. Each heatwave lasted nine days, where during the first three days, temperature was increasing, reaching a peak phase on the fourth day

and staying for four days at the peak phase, followed by two days of cooling. The peak phase of the first two heatwaves was 3.6°C, while that of the third one was 5.2°C higher than the control treatment (for details see Pansch et al., 2018). For more details on the heating system of the mesocosm experiment, see Wahl et al. (2015). In the case of the laboratory experiment, temperature inside the experimental tanks was daily manually adjusted using aquaria heaters submerged into the water baths (Aqua Medic titanium heaters 100W), with submersed pumps insuring homogenous mixing of water inside the baths (Figures 1 and 2). The temperature inside each water bath

was monitored every 30 min throughout the experiment using temperature loggers (HOBO Pendant® Temperature Data Logger).

2.4 | Statistical Analysis

First, we calculated the percentage of population growth for each replicate of each treatment in each experimental type following the equation:

$$R = 100 \times k \quad (1)$$

where R is the per cent change per unit time (i.e. growth rate) and k is the fractional change per unit time (Bartlett, 1993). The k was calculated as follows:

$$k = (N_{\text{end}} - N_{\text{beg}}) / N_{\text{beg}} \quad (2)$$

with N_{end} being the number of individuals at the end of the experiment and N_{beg} being the number of individuals at the beginning of the experiment. Then, variation in percentage of population growth between the species within experimental type was compared using two-way factorial analysis of variance (ANOVA) where percentage of population growth was the dependent variable, and species (i.e. *G. locusta* and *G. salinus*) and heatwave treatments (i.e. control, one heatwave and three heatwaves) were the independent variables. Two separate ANOVAs were conducted, one for each type of experiment. We did not statistically compare the two types of experiments due to potential confounds, such as time, as the experiments were conducted in different years. For all comparisons, the assumptions of normality and homogeneity of variances were confirmed using the Shapiro–Wilk's W test and Fligner–Killeen test, respectively. Statistical analyses were performed using the R software, version 3.5.2 (R Core Team, 2018). Data visualization was conducted by “ggpubr” and “ggplot2” packages in R (Kassambara, 2018; Wickham, 2016).

3 | RESULTS

In the mesocosm experiment, there was no significant difference in the percentage of population growth between the two species (ANOVA, $F(1,18) = 3.563$, $p = .075$), though *G. locusta* had slightly higher mean number of individuals at the end of the experiment in all treatments when compared to *G. salinus* (i.e. mean and standard deviation were 485.3 ± 162.7 , 664.5 ± 162.8 and 560.5 ± 353.1 for *G. locusta*, and 336.0 ± 244.8 , 427.0 ± 144.9 and 451.3 ± 132.8 for *G. salinus* in the control, one heatwave and three heatwave treatment, respectively; Table 1). In contrast, in the laboratory experiment, the percentage of population growth between the two species was significantly different (ANOVA, $F(1,18) = 23.499$, $p < .001$), with *G. salinus* having significantly higher relative population growth across all treatments. While number of individuals of *G. salinus* increased by an order of magnitude during the laboratory experiment, in the case

of *G. locusta*, there was either no change between the number of individuals at the beginning and that at the end of the experiment, or the number decreased resulting in the negative growth of the population (Table 1; Figure 3). At the end of the laboratory experiment, the mean and standard deviation of *G. locusta* individuals in the control, one heatwave and three heatwave treatment were 30.3 ± 17.8 , 27.8 ± 10.6 and 18.0 ± 8.5 , respectively, while those of *G. salinus* was 137.0 ± 44.9 , 152.3 ± 88.3 and 114.8 ± 89.5 , respectively. Finally, neither the one heatwave nor three heatwave treatment demonstrated any significant change in the percentage of population growth for either of the species in either experimental type (ANOVA, $F(2,18) = 0.838$, $p = .448$ and ANOVA, $F(2,18) = 0.392$, $p = .681$ for the mesocosm and laboratory experiment, respectively).

4 | DISCUSSION

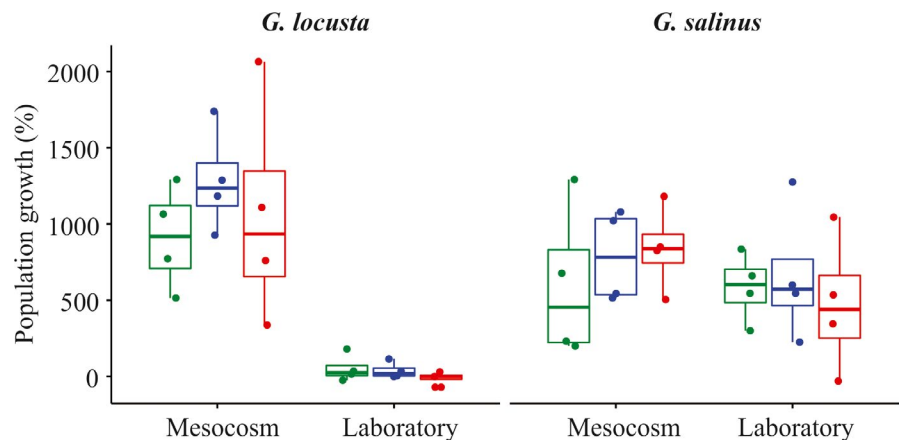
While laboratory experiments are mostly focused on specific physiological and ecological responses to environmental changes of individuals or a single species, mesocosms embrace a higher complexity by including assemblages of a population subset which increases the possibility of biological interactions and “ecosystem realism” (Stewart et al., 2013; Widdicombe et al., 2010). However, there is still considerable uncertainty with regard to both types of experiments, and to which extent their results can be extrapolated and generalized with confidence. Our comparative assessment of mesocosm with laboratory experiment revealed that while for one species the results were similar independently of the experimental type, for the other species, the larger area of the mesocosm accompanied with inclusion of the community benefited the species' growth rate, demonstrating stronger performance in the mesocosm than in the laboratory experiment. Though, we acknowledge here that our study design did not allow to distinguish if the scale or ecological complexity of the experiments, or both, caused the observed discrepancy between the two types of experiments. We also acknowledge that we did not statistically compare the experiments due to potential time confounding factor as the experiments were conducted in different years, but we qualitatively compared the results. However, at the same time, our results revealed no difference in the heatwave impacts on any of the tested species independently of which experimental type was used.

The potential impacts of climate change on coastal marine environments and freshwater ecosystems have been extensively studied since the early 1990s where most studies have mainly focused on the species level (Harley et al., 2006; Wrona et al., 2006 and references therein). Lately, much more information using mesocosm experiments became available, which has been used, in some way, to calibrate and confirm that laboratory experiments properly represent natural ecosystems and their interactions (Schindler, 1998). Interestingly, our results have led us to two different conclusions for the two studied species that were tested using different experimental types. Independently of the heatwave treatment, *G. salinus* results were similar regardless of the experimental type,

TABLE 1 Number of individuals in each replicate for both species (*Gammarus locusta* and *G. salinus*) at the beginning and at the end of both experiments (i.e. mesocosm and laboratory) in all three treatments (i.e. control, one heatwave and three heatwaves), and the respective population growth (R)

Control				One heatwave				Three heatwaves					
Gammarus locusta													
Mesocosm experiment													
Beginning	48	48	48	48	48	48	48	48	48	48	48	48	48
End	559	247	371	620	493	616	666	883	1,039	210	413	580	
R	1,065	515	773	1,292	927	1,183	1,288	1,740	2065	338	760	1,108	
Laboratory experiment													
Beginning	20	20	20	20	20	20	20	20	20	20	20	20	20
End	27	56	15	23	20	27	43	21	20	20	6	26	
R	35	180	-25	15	0	35	115	5	0	0	-70	30	
Gammarus salinus													
Mesocosm experiment													
Beginning	48	48	48	48	48	48	48	48	48	48	48	48	48
End	373	668	159	144	538	309	566	295	444	290	615	456	
R	677	1,292	231	200	1,021	544	1,079	515	825	504	1,181	850	
Laboratory experiment													
Beginning	20	20	20	20	20	20	20	20	20	20	20	20	20
End	152	80	187	129	140	65	275	129	89	127	229	14	
R	660	300	835	545	600	225	1,275	545	345	535	1,045	-30	

FIGURE 3 Population growth (%) of individuals of *Gammarus locusta* and *G. salinus* in both mesocosm and laboratory experiments. Experimental treatments are depicted in green, blue and red for control, one heatwave and three heatwaves, respectively. The boxes represent upper and lower quartiles with median line, while dots represent the separate replicates



while for *G. locusta*, our study revealed differences between the two experimental types, with much poorer performance of individuals under laboratory conditions. Therefore, if one would study competition between these two species under current and/or future global warming scenarios under laboratory conditions, they could conclude that *G. salinus* would outcompete, or reduce population size, of *G. locusta*. Considering that settings in our study were exactly the same for both species in both experimental types, we believe that *G. locusta* is more sensitive to laboratory setting than *G. salinus*. Yet, our study design does not differentiate if the scale or ecological complexity of the experiments, or both, were responsible for the observed results. Under low salinity environments, several laboratory studies, for instance Bulnheim (1979) and Paiva et al. (2018), have determined that *G. locusta* is the most

sensitive species among the Baltic gammarids with the lowest capacity to survive. In addition, this species reveals a much higher oxygen intake when exposed to such stress in comparison with other species, which seems to explain its absence from polluted areas (Bulnheim, 1979; Costa & Costa, 2000). Another explanation could be that the small space of the tanks used in the laboratory experiment in addition to the absence of natural predators and food availability have triggered possible fighting and cannibalistic behaviour within and among species. Our laboratory experiment has been started by two orders of magnitude higher density of each species than those in the mesocosm experiment. Therefore, density-dependent effects experienced in the laboratory conditions may have been different from those in the mesocosms, causing bias in the observed results. As reported by Dick (1995),

Gammarus spp. are omnivores with tendency to strong predatory activities, especially on susceptible individuals such as juveniles, and might prey on congeneric in order to get nutritional profits. Though cannibalism occurs under natural and semi-natural conditions, decreased structural habitat complexity might increase predatory activity (Christie & Kraufvelin, 2004; Dick, 1995; Dick & Platvoet, 1996; Macneil & Prenter, 2000). In addition, cannibalism and intraguild predation have been suggested as regulation factors when population density is too high (Christie & Kraufvelin, 2004). Consequently, though artificial refuges were provided in our laboratory experiment, these might have been ineffective due to the high density of individuals and consequent density-dependent effects since the two studied species are potential competitors for both space and food. As at the end of the experiment, we observed the majority of individuals of *G. locusta* being adults, as well as unchanged abundance of *G. locusta* between the beginning and the end of the experiment, we believe that the predation on its juveniles occurred, affecting the population growth rate of the species. Finally, due to unpredictable behaviour of species in highly artificial environments, such as laboratory experiments, we emphasize a necessity of great caution when testing and interpreting results on species interactions and/or impact on each other.

While the overall aim of experiments is to provide essential knowledge of current and future threats to diverse communities or to study species and/or communities in general, the conducted experiments may not always be representative of natural systems (Cooke et al., 2017; Kraufvelin et al., 2006, 2010, 2020; Widdicombe et al., 2010). In fact, our study raised an important question considering the reliability of our own laboratory experiment and laboratory types of experiments in general. In the mesocosm experiment, half of the tested species demonstrated tolerance to heatwaves, which included both *G. locusta* and *G. salinus*, with only few species responding strongly negatively (see Pansch et al., 2018). However, in apparent contrast to the observed lack of sensitivity to heatwaves of *G. locusta* in both experimental types in our study where during the last heatwave temperature reached 25.2°C, previous laboratory studies have found high mortality rate of this species above 20°C and 22°C and suggested future global warming scenario exceeding the thermal limit of the species (Cardoso et al., 2018; Neuparth et al., 2002). Similarly, *Marenzelleria viridis*, a successful invader in Baltic waters, revealed a positive effect of heatwave treatments on both its biomass and abundance in our mesocosm experiment (see Pansch et al., 2018). However, Bochert et al. (1996), using laboratory experiments, found a temperature of 20°C to be too high for a proper development of the species suggesting an abnormal growth during larval phase. Therefore, while the laboratory experiment of our study produced similar results to the mesocosm experiment when testing tolerance of species to heatwaves, our experiments were contrasting findings of previously conducted laboratory experiments testing resilience of those species using constantly elevated temperatures (Bochert et al., 1996; Cardoso

et al., 2018; Neuparth et al., 2002; Pansch et al., 2018). As the results of laboratory experiments are not being consistent, and sometimes contradict the results of mesocosm experiments, we stress a need of conducting more comparative studies between mesocosm and laboratory experiments to determine to which extent they are comparable and represent reality in nature. Though, as a result of this study, we trust that the use of mesocosm instead of laboratory experiment is advocated whenever possible, as available space and/or community interactions are of paramount importance for species fitness.

Though some organisms may, or may not, show resistance in controlled laboratory experiments when exposed to a single, or multiple stressors, their sensitivity may change when exposed to other factors, such as the complex physical components and biotic interactions of the natural environments (Sommer et al., 2012). Although such laboratory experiments may improve our knowledge on the physiological response of the individuals, they are not a true replication of what occurs in nature. Actually, our study strongly indicated that the same species may respond differently when tested at high density and isolated from a community than when at lower density and in the presence of a subset of a community, confirming recent finding by Wahl et al. (2020). Furthermore, inconsistency in results among laboratory experiments complicates the extrapolations and generalization of the laboratory results even more. Our findings indicate the importance of scale, density, biotic interactions and complexity of natural environments in buffering, or boosting, the direct effects of environmental stress on organisms. Therefore, we urge the use of mesocosm experiments whenever possible, and emphasize a necessity of great carefulness when interpreting and generalizing the results of laboratory experiments.

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PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ddi.13213>.

DATA AVAILABILITY STATEMENT

The primary data set containing experimental results is available at Dryad: <https://doi.org/10.5061/dryad.gtht76hkf>, and Pangaea: <https://doi.org/10.1594/pangaea.888578>.

ORCID

Elizabeta Briski  <https://orcid.org/0000-0003-1896-3860>

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BIOSKETCH

The authors are marine biologists who work across multiple disciplines focusing on the impacts of global climate change and human activities on the oceans in general. More detailed information about each author can be found on the following websites.

<http://pansch-research.com/>

<http://elizabethabriski.yolasite.com/>

<http://www.ipn.uni-kiel.de/en/the-ipn/departments/chemistry-education/staff/brennecke-dennis>

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