Urban environments promote adaptation to multiple stressors

Elizabeta Briski[1] (ebriski@geomar.de), Louisa Langrehr[1,2](Louisa.Langrehr@gmx.net), Syrmalenia G. Kotronaki[1,3](sirmalenia.k@gmail.com), Alena Sidow[1](alena.sidow@web.de), Cindy Giselle Martinez Reyes[1](cgmartinez96@gmail.com), Antonios Geropoulos[4](bio2822@hotmail.com), Gregor Steffen[1](phat@gmx.com), Nora Theurich[1,5](nora.theurich@gmail.com), James W.E. Dickey[1,6,7,8](jamesdickey03@gmail.com), Jasmin C. Hütt[1](jashuett@gmail.com), Phillip J. Haubrock[9,10,11](phillip.haubrock@senckenberg.de), Ismael Soto[9](isma-sa@hotmail.com), Antonín Kouba[9](akouba@frov.jcu.cz), and Ross N. Cuthbert[1,12](r.cuthbert@qub.ac.uk)

[1]GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel, Wischhofstraße 1-3, 24148 Kiel, Germany

[2]Carl von Ossietzky Universität Oldenburg, Ammerländer Heerstraße 114-118, 26129 Oldenburg, Germany

[3] Department of Biological Sciences, Lehigh University, Bethlehem PA 18015, USA

[4] Faculty of Science and Technology, Biology Department University of Crete, Marine Ecology Lab, Vasilika Vouton, 70013 Heraklio, Crete, Greece, P.O.Box 2208

[5]Christian-Albrechts-Universität zu Kiel Christian-Albrechts-Platz 4, 24118 Kiel, Germany

[6]Berlin-Brandenburg Institute of Advanced Biodiversity Research, 14195 Berlin, Germany
[7]Freie Universität Berlin, Institute of Biology, 14195 Berlin, Germany
[8]Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), 12587 Berlin, Germany
[9]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic
[10]Senckenberg Research Institute and Natural History Museum Frankfurt, Department of River Ecology and Conservation, Gelnhausen, Germany
[11]Center for Applied Mathematics and Bioinformatics, Department of Mathematics and Natural Sciences, Gulf University for Science and Technology, Hawally, Kuwait
[12]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, Northern Ireland, UK

Short title: Urban adaptations to stress

Keywords: aquatic ecosystem; environmental change; land use; pCO_2; salinity; temperature

Type of article: Letter

Number of words in the abstract: 150

Number of words in the main text: 4202

Number of references: 78

Number of figures: 4

Number of tables: 1

***Correspondence:** Elizabeta Briski, GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel, Wischhofstraße 1-3, 24148 Kiel, Germany; tel. +49 431 600 1589, e-mail ebriski@geomar.de

AUTHOR CONTRIBUTIONS:

EB: Conceptualization. EB, LL, SGK, AS, CMR, AG, GS, NT, JWED, JCH and RNC: Investigation. EB, PJH, IS, AK and RNC: Formal analysis and visualisation. EB and RNC: Writing – Original draft. All authors: Writing – review & editing. RNC and EB: Funding acquisition.

DATA AVAILABILITY STATEMENT

Raw data supporting the findings of this study is available at Drayad: https://doi.org/10.5061/dryad.3tx95x6qx and R code used for all of the GLMs at Zenedo: https://doi.org/10.5281/zenodo.14616924.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICS APPROVAL

Ethical approval was not required for the nature of this work.

ABSTRACT (150 words)

Anthropogenic activities have drastically changed environmental conditions worldwide, negatively impacting biodiversity and ecosystem services. At the same time, the majority of the human population lives in urban areas that are greatly altered from natural habitats. Nevertheless, many species thrive in urban environments. To improve our knowledge of evolution and adaptation in these anthropogenically impacted habitats, we conducted the widest series of stress experiments to date with three marine taxa: one mussel and two gammarid species. We compared intraspecific populations from protected and human-altered habitats to determine their tolerance to salinity, temperature and partial pressure of CO_2 in water (pCO_2) regimes. Populations from impacted habitats typically outperformed protected habitat populations, with individuals from the most impacted habitat being the most robust. We propose that urban populations are adapting to life in disturbed environments — this adaptation concurrently promotes more resilient rescue populations but potentially confers increased invasion risk from non-native species.

INTRODUCTION

The Earth and its environments are being rapidly changed by increasing human populations and economic development (Hawkins, 2012). Anthropogenic activities, including burning of fossil fuels and deforestation, among others, have triggered climate change, pollution, soil erosion, and habitat destruction (Hawkins, 2012; IPCC, 2014). The introduction of non-native species (i.e., species moved by human activity outside their native region) is also prominent due to the increasing global trade and transport of materials (Hulme, 2009; Sardain et al., 2019). Subsequently, these changes have dramatically affected numerous ecosystems worldwide, leading to biotic homogenization, decreases in biodiversity, and occasionally, to losses of ecosystem functioning and services (Olden et al., 2004; Hawkins, 2012; IPCC, 2014; Linders et al., 2019; Pyšek et al., 2020).

More than half of the global human population presently lives in urban areas, with these numbers expected to be double by 2050 (Johnson & Munshi-South, 2017; United Nations Statistics Division, 2017). This urbanized world consists of dense settlements made of buildings, roads, and other supporting infrastructures, with urban habitats significantly altered from natural ones (Johnson & Munshi-South, 2017). As a consequence of rising urbanization, environments in cities exhibit altered microclimates, which trend towards higher temperatures, alongside elevated air, water, noise, and light pollution (LLUR, 2001; Grimm et al., 2008; Nikulina et al., 2008). They also experience increased habitat fragmentation (Dobbs et al., 2017), lower diversity and abundance of native species, high numbers of non-native species (Aronston et al., 2014; McKinney et al., 2008), and lower phylogenetic diversity within communities (Knapp et al., 2012; Sol et al., 2017). Still, urban environments are successfully exploited by many non-human species (Johnson & Munshi-South, 2017; Perry & Göttert, 2024). Pigeons, peregrine falcons, ducks, carps, and goldfish are just some of the examples of those that thrive in urban habitats.

While rates of evolution had been deemed to be too slow to have relevance to urbanization, recent research across a wide variety of taxa has demonstrated that organisms can indeed evolve rapidly in response to urban environments (Alberti et al., 2017; Johnson & Munshi-South, 2017; Caizergues et al., 2018; Santangelo et al., 2018; Thompson et al., 2018; Borden & Flory, 2021; Perry & Göttert, 2024). Evolutionary changes have been observed in as little as two generations (Kinnison & Hendry, 2001), and can include behavioral, morphological, and physiological adaptations, or even speciation, in response to altered environmental conditions, novel resources and different physical habitats, among others (Giraudeau et al., 2014; Winchell et al., 2016; Martin et al., 2019; Borden & Flory, 2021; Ålund et al., 20204). These changes may be particularly rapid in urban environments, where significant alterations to numerous abiotic and biotic factors are simultaneous and often predictable. Therefore, studies of adaptation in urban environments may be ideally placed for testing wider evolutionary theory across taxa (McKinney, 2002; Grimm et al., 2008), particularly in the context of multiple stressors (Todgham et al. 2005). In turn, understanding the adaptation potential of diverse taxa, as well as ecosystem resilience to anthropogenic stressors, urbanization, and global change, are of paramount importance to inform conservation actions, even in natural habitats.

In the last two decades, whereas research into the effects of global change on ecosystems has grown substantially, eco-evolutionary dynamics in urban environments have been neglected outside of the terrestrial realm (Johnson & Munshi-South, 2017; Santangelo et al., 2018; Thompson et al., 2018; Alter et al., 2020; Borden & Flory, 2021; Perry & Göttert, 2024). Marine ecosystems have been particularly overlooked in terms of urban evolution, but provide exceptional model systems considering the presence of multiple stressors and clear disturbance gradients (e.g., harbours versus protected areas). To improve our knowledge on evolution and adaptation potential of taxa in urban and anthropogenically impacted habitats, we conducted a series of laboratory experiments among populations of one mussel and two gammarid species collected at protected and human-altered habitats in the Baltic Sea, to determine their tolerance (i.e., plasticity) to diverse levels of salinity, temperature, and pCO_2 (i.e., partial pressure of CO_2 in water). Taking into account the concurrent evolution of adaptation to heterologous stressors (Todgham et al. 2005), the overall aim of this study was to test whether populations inhabiting human-altered habitats are better adapted to anthropogenic stressors, including climate change, than populations from protected ones. We tested the null hypothesis that there is no difference in stress resistance between populations inhabiting human-altered and protected habitats.

MATERIALS AND METHODS

Experimental organisms and collection sites

Individuals of populations of one mussel (i.e., *Mytilus* sp.) and two gammarid species (i.e., *Gammarus locusta* and *G. salinus*) were collected in protected and human-altered (i.e., impacted) habitats in the Baltic Sea in Germany (Figure 1). Only hybrids of *M. edulis* and *M. trossulus* are present at our sampling locations (Knöbel et al., 2021); therefore, we refer to the mussel species used as *Mytilus* sp. In the case of gammarids, each individual was morphologically identified according to Köhn & Gosselck (1989) and Zettler & Zettler (2017). All three taxa used in the experiments are native to the Baltic Sea (Springer & Crespi, 2007; Gaitán-Espitia et al., 2016; Cuthbert et al., 2020; Briski et al., 2024). While both gammarid species (alongside ~99% of global biodiversity) do not yet have an invasion record (Cuthbert et al., 2020; Briski et al., 2024), the mussel *M. edulis* is non-native in Southern Europe, Asia, and North America (Springer & Crespi, 2007; Gaitán-Espitia et al., 2016; Briski et al., 2024); *Mytilus trossulus* is non-native in the Black Sea (Briski et al., 2024). Though, we emphasize that *Mytilus* spp. taxonomy and distribution are complex due to their cryptic morphology and the frequent occurrence of hybridization between species (Springer & Crespi, 2007; Gaitán-Espitia et al., 2016). The protected habitat, Schleimünde (Maasholm), has been a nature reserve since 1972 (Verein Jordsand zum Schutze der Seevögel und der Natur e.V., 2019). According to its 'EU Water Framework Directive' status, the Schlei system is in a good chemical condition (LLUR, 2001; Alpert et al., 2015). Though, we emphasize there were no chemical concentrations reported. The human-altered habitat was the Kiel fjord, with three locations sampled, depending on the species: i) downtown Kiel; ii) Kiel canal; and iii) Falckenstein beach (Figure 1). Downtown Kiel is a completely artificial habitat with concrete structures, while the entire Kiel fjord, including downtown Kiel, is heavily impacted by the shipping industry and tourism, and exposed to extensive pollution including heavy metals such as copper, zinc, tin, lead, as well as tributyltin (LLUR, 2001; Nikulina et al., 2008). The levels of copper, zinc, tin, and lead were higher deeper in the Fjord, with values increasing from 40, 80, 4 and 40 mg/kg at Falckenstein beach to 100, 240, 16 and 100 mg/kg at downtown Kiel, respectively (Nikulina et al., 2008).

Individuals of at least two populations of each species were collected: one population in the protected habitat and one or more in a human-altered habitat. In the case of *G. salinus*, three populations were sampled in human-altered habitats alongside a protected one. In this case, we aimed to determine if there was a difference in performance among populations adapted to various human-altered habitats along a perceived gradient of anthropogenic influence. Species were collected from May 2016 until January 2021, depending on when a particular species/population experiment was conducted as well as our sampling logistics. In the majority of cases, experimentation was in the same year and season for population comparisons from an individual species (Table S1). After collection, the animals were transported to the laboratories at GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel in their ambient water and acclimatized for at least two weeks before the experiments commenced. During the acclimatization period, animals were kept in their ambient water in 56 L glass aquaria in the laboratory environment (separately per species/population; Table S1). Each aquarium was aerated through a spongy filtration system to maintain water quality. The light regime was 12:12h light and dark. Mussels were fed every three days with commercial live marine phytoplankton (Premium Reef Blend, Sustainable Aquatics), while gammarids were fed weekly with a mixture of commercial crustacean food (Tetra Mix, Tetra Crusta, and Dr. Shrimp Healthy).

Experimental design

Two different types of experiments were conducted for each population of each species, with two crossed stressors: i) temperature – pCO_2 (i.e., partial pressure of CO_2 in water); and ii) temperature – salinity (Table 1). In the case of the temperature – pCO_2 experiments, a fully crossed factorial design consisting of two temperature levels and four pCO_2 levels was performed (Table 1). The temperature – salinity experiments consisted of three temperature and two salinity levels (Table 1). The temperature and pCO_2 values were based on current and future predicted values for the Baltic Sea, as well as occasional measurements in Kiel fjord due to heat waves and seasonal upwelling events (Thompsen et al., 2012; Pansch et al., 2018; Meier et al. 2022). The lower salinity value was based on the regularly measured salinity of the sampled locations, with the higher salinity constituting a rare but ecologically relevant level measured during winter months at the impacted habitats (Kazanavičiūtė et al., 2024; Briski, personal observations).

In both types of these multiple-stressor experiments with factorial stressor arrangements, following the acclimatization period to the laboratory environment, the stress tests were conducted using ten mean-size adult individuals per replicate; except in two experiments when eight and nine individuals were used due to a lack of individuals (i.e., in a temperature – pCO_2 experiment with *Mytilus* sp. from Schleimünde and a temperature – salinity experiment with *G. locusta* from Schleimünde, respectively). The experimental individuals were transferred from the acclimation conditions to the experimental conditions without gradual adjustments (see Tables 1 and S1). Three replicates were conducted for each treatment. Mussel experiments were performed in 5 L aquaria, whereas those for gammarids were in 2 L aquaria containing artificial habitat structures to provide complexity of habitat. One aquarium represented one replicate per treatment. The experiments lasted for 30 days, with mortality checked daily. At that time, dead individuals, molted exoskeletons (in the case of gammarids), and newly emerged juveniles (in the case of gammarids) were removed. Light and feeding regimes were kept the same as during the acclimation period. The experimental conditions and treatments differed among species/experiments, but analyses were performed within species (i.e., comparisons were made among intraspecific populations per experiment), and therefore our approach was not confounded by these disparities.

In the first type of experiment, i.e., temperature – pCO_2 experiments, the aquaria were placed in water baths at the desired temperature, with pCO_2 levels being randomly assigned within each water bath, and water continuously infused with its determined pCO_2 level using an air stone. The water used to fill the tanks was filtered from the surrounding area of the institute (filtered through 5 µm filter). The aquaria were sealed using a plastic cover to stabilize the pCO_2 levels. Throughout the experiments, the salinity was maintained at the ambient condition for each species/population to reduce any additional stress. Temperature, pH (WTW pH 3110 with a SenTix 81 pH electrode), and salinity (WTW Cond 3110 with a Tetracon 325 probe) were recorded daily. Every ten days, 50% of the water was exchanged with previously prepared water to ensure the desired temperature and pCO_2 conditions were maintained. In the second type of experiment, i.e., temperature – salinity, the aquaria were placed in water baths at the desired temperature, with salinity levels being randomly assigned within each water bath. The aquaria were constantly aerated. Salinity and temperature were checked daily, with a 50% water change done every ten days using previously prepared water to ensure the desired salinity and temperature conditions.

Statistical analyses and visualization

Mortality curves were constructed for each population for each treatment, described by the equation (Briski et al. 2008, 2011):

$$y=100/[1+e^{-Z(t-Q)}] \qquad (1)$$

where Z is the slope of the mortality rate, t is time and Q is the onset of mortality. All curves were constructed using S-Plus 6.1 (S-Plus ® 6.1, 2002; Insightful Corp., Seattle, WA, USA). Raw data supporting the findings of this study is available at Dryad under doi: https://doi.org/10.5061/dryad.3tx95x6qx.

For each species and experiment separately, generalized linear models with binomial error distributions and log links were used to examine survival rates among populations, temperatures, and either salinities or pCO_2 levels at day-15 (mid-point) and day-30 (end-point).

Accordingly, twelve models were built in total (3 species × 2 experimental contexts [temperature + pCO2/salinity] × 2 time points). Non-significant terms were removed backward and stepwise to obtain the most parsimonious models. In cases of complete separation (i.e., total survival or mortality in an experimental group), bias reductions were employed to fit the model to the data (Kosmidis, 2020). Residuals were checked for overdispersion via comparisons of simulated and observed residual distributions (Hartig, 2020). Analysis of deviance with type-3 sums of squares was used to compute coefficients where there were more than two populations in a given model (i.e., models considering *G. salinus*). Tukey comparisons were used to examine significant effects and interactions pairwise post-hoc (Lenth, 2020). Significance was inferred at $p < 0.05$, and generalized linear models were fit in R (R Core Development Team, 2022).

RESULTS

***Mytilus* sp.**

In response to temperature and pCO_2 gradients, significant differences for *Mytilus* sp. were observed between populations from human-altered and protected habitats (Table S2), across both time points (Figure 2). Mortality in the population from human-altered habitat tended to peak at the highest pCO_2 level, while mortality in the population from protected habitat peaked at the lowest pCO_2 levels, driving a significant two-way interaction between population and pCO_2 (Table S2). Significant population-level differences were also observed between groups from human-altered and protected habitats in response to the experimental temperature and salinity gradients (Table S2), with the protected site population exhibiting greater mortality in all groups at both time points — except at the highest temperature and salinity (Figure 2). In that treatment, there was a much more rapid increase in mortality in the population from the human-altered habitat, where it overtook the population from the protected habitat, as evidenced by a significant three-way interaction term (Table S2).

Gammarus locusta

In response to temperature and pCO_2 gradients, population-level responses by *G. locusta* were significantly influenced by pCO_2 at both time points (Table S3), with the population from the protected habitat tending to exhibit higher mortality (Figure 3). Mortality rates of the population from human-altered habitat tended to decrease with increasing pCO_2, whereas the opposite was observed for the population from protected habitat (Figure 3). Therefore, population-level mortalities were most divergent at the highest pCO_2 levels. In contrast, no significant inter-population differences were observed between those from protected and human-altered habitats across temperature and salinity groups at either time point (Table S3). For both populations, mortality significantly increased with temperature and with falling salinity (Figure 3).

Gammarus salinus

In the temperature and pCO_2 experiments, *G. salinus* exhibited significant differences according to temperature and population in interaction at day-15 independently of pCO_2 (Table S4). Mortality rates at day-15 in the population from the most impacted human-altered habitat (i.e., downtown Kiel) decreased with warming, while the other two populations (i.e., Kiel canal and Falckenstein beach) exhibited higher mortality with greater temperature (Figure 4). At day-30, a significant three-way interaction emerged, whereby differences between populations from the most impacted human-altered habitat (i.e., downtown Kiel) and less impacted (i.e., Kiel canal) and protected habitats (i.e., Schleimünde) were greatest at the lower temperature and higher

pCO_2 levels (Table S4; Figure 4). In the salinity and temperature experiments, inter-population responses were influenced by temperature and salinity in interaction at day-15, but not at day-30 (Table S4). Mortality rates of all populations except the one from the protected habitat (i.e., Schleimünde) increased with greater temperature and at the lower salinity at day-15. These differences were less obvious by day-30, with mortality rates for human-altered and protected habitat population differences more consistent (Figure 4). The population from the most impacted human-altered habitat (i.e., downtown Kiel) and that from one of the less impacted (i.e., Falckenstein beach) had significantly lower mortality, with differences most pronounced between populations from those sites and populations from the other less impacted (i.e., Kiel canal) and protected habitats (i.e., Schleimünde) at the highest temperature and lower salinity. The population from the most impacted human-altered habitat had significantly lower mortality than all other populations overall.

DISCUSSION

Stresses associated with urban areas are pervasive, however, empirical testing of hypotheses linked to adaptation to anthropogenically modified habitats has remained scarce. Our study tested the performance of different populations of the same species of mussels and gammarids inhabiting impacted and protected habitats, and demonstrated that populations from impacted habitats typically performed better under a broad range of environmental conditions than those from the protected habitat. The populations from protected habitat were consistently less robust, or plastic, to multiple anthropogenic stressors. Based on stress performance and phenotypic plasticity, our results indicate that populations in urban, impacted habitats are adapting to life in anthropogenic environments.

Our study determined distinct differences between populations from impacted and protected habitats, with impacted environments producing populations more tolerant to multiple abiotic stressors. However, there was also an apparent stress response gradient within populations from urban areas of differing degrees of habitat alteration. This suggests that the development of resistance along anthropogenic gradients is continuous and not binary. Indeed, in the case of both gammarid species, the outer locations in the Kiel fjord at both the Falckenstein beach and Kiel canal sites produced populations less robust to stressed environments, with *G. salinus* exhibiting the highest tolerance at the most impacted and innermost downtown Kiel site. Falckenstein beach is sandy, lacking concrete and areas of altered habitat, however, the location is still exposed to frequent shipping transits throughout the year and the tourist industry in summer months. Kiel canal is located deeper in the Kiel fjord and contains a large amount of concrete as well as ship traffic. The most impacted site, downtown Kiel, is a primarily concrete artificial habitat, exposed to diverse anthropogenic impacts from the shipping industry, high heavy metal concentrations, as well as light and noise pollution and tourism (LLUR, 2001; Nikulina et al., 2008). Consequently, while specific environmental conditions driving adaptation require elucidation at these sites, it seems that the protected nature of a habitat does not play the main role in shaping the robustness of taxa. Rather, resistance is determined by the intensity of stressors present in a habitat, likely linked to the distance of the habitat from our cities and industries, and possibly the potential for taxa to move to surrounding refugia. While we made these inferences based on co-occuring temperature, pCO_2, and salinity stressors, which are pervasive in the Baltic Sea context, we acknowledge that other stressors linked to different forms of pollution (e.g., noise, light, heavy metals) require examination. Nevertheless, cross-tolerance among heterologous stressors is possible (Todgham et al., 2005), with the ability of one stressor to increase tolerance to a second heterologous stressor, and therefore our stressor selection could approximate adaptive responses to other stressors.

As we did not test the mussel population from Falckenstein beach or the Kiel canal, but only from downtown Kiel, our study cannot show if mussels from these ‘intermediate’ impacted sites demonstrate the same pattern as gammarids. Nevertheless, mussels are sessile filter feeders and once their larvae are settled on a substrate, it is almost impossible for individuals to move further to more hospitable areas and seek refugia (Ruppert et al., 2003). In contrast, gammarids are mobile detrivores that can actively retreat to less hostile environments when needed (Ruppert et al., 2003; Gerhardt et al., 2011). Therefore, we speculate that the mussel population from Falckenstein beach might have been more robust to stressors than the population from the protected habitat. We would speculate the same for other sessile organisms, such as macroalgae and seagrass. We encourage future studies to test our assumptions and assess taxonomic differences in stress resistance and adaptation among taxonomic groups across varyingly impacted habitats.

Occurrences of anthropogenically impacted populations underperforming individuals from the protected site were rare. However, in the highest temperature – salinity treatment, the population of *Mytilus* sp. from the impacted habitat exhibited a significantly faster mortality rate than the population from the protected habitat. As all replicates demonstrated similarly high mortality rates, we believe that there was no laboratory effect causing this rapid mortality of the population, but it was probably due to the combination of stressors being too impactful and exceeding the threshold of the population’s stress tolerance. Similarly, in the case of *G. locusta* in the combination of the higher temperature (i.e., 24 °C) with all pCO_2 levels, the mortality of both populations was remarkably fast, with no survival displayed after two weeks. These examples further support population-level nuances while also highlighting that even populations from anthropogenically-stressed habitats are susceptible to abiotic limits. Hostile environments place extra pressure on organisms, as modifications from optimal environments require additional energetic costs because of, for example, a higher rate of osmoregulation in suboptimal salinity regimes and an increase in metabolic rate with elevated temperatures, leading to a decrease in organisms' fitness (Gillooly et al., 2001; Evens, 2008; Pörtner, 2010; Bruno et al., 2015; Rivera-Ingraham & Lignot, 2017). In the case of highly hostile environments where the effects of stressors go beyond the threshold of the population's stress tolerance, those additional energetic costs are disproportionately high, and eventually lead to mortality (Gillooly et al., 2001; Evens, 2008; Pörtner, 2010; Bruno et al., 2015; Rivera-Ingraham & Lignot, 2017). Future research should elucidate whether gradual acclimatization to these stressors can mediate levels of mortality within the laboratory through comparison with acute stress exposures. The resilience to the degree of variability in environmental stressors over time should also be examined among populations (Morón Lugo et al., 2020).

Furthermore, the 'anthropogenically induced adaptation to invade' (AIAI) hypothesis and urban evolution concept propose that contemporary adaptation of populations to human-altered habitats (e.g., cities, shipping ports, fragmented urban habitats) within the native range can promote establishment success and increase levels of impact in non-native regions (Hufbauer et al., 2012; Borden & Flory, 2021; Perry & Göttert, 2024). Indeed, human-altered habitats have relatively similar environmental conditions globally and are increasingly prevalent with human population growth, especially in cities (Grimm et al., 2008). Moreover, most long-distance transport happens between two urban habitats (e.g., between two shipping ports; Hulme, 2009; Kaluza et al., 2010; Sardain et al., 2019), and thus, the presence of adapted populations in the human-altered habitats can experience increased transport probability, higher chances of surviving the harsh transport conditions, and a reduced need for further adaptation in the non-native regions following introduction (Hufbauer et al., 2012; Lockwood et al., 2013; Briski et al., 2013, 2014, 2018). Finally, due to broader phenotypic plasticity of those populations, they would also have better chances for further adaptation and evolution in new habitats, particularly if new habitats are more variable and different from their native ones (Buczkowski, 2010; Dlugosch et al., 2015; Cadotte et al., 2017; van Kleunen et al., 2018; Westneat et al., 2019; Haubrock et al., 2021). Therefore, adaptations to anthropogenic and urban stressors could contribute substantially to invasion success of non-native species across all stages of the invasion process – uptake in a native region, transport, introduction, establishment, and spread/impact.

These altered ecosystems can also act as experimental ‘time machines’ for studying adaptation potential to predicted future global changes, as these habitats, for example, are often warmer and have higher levels of CO_2 than surrounding natural areas (Grimm et al., 2008; Lahr et al., 2018). In our study area, Kiel fjord, for example, is characterized by strong seasonal pCO_2, variability (Thompsen et al., 2012). Similarly, large estuaries, which are heavily inhabited by humans and contain numerous shipping ports, such as the Saint Lawrence estuary in Canada, or the Chesapeake Bay and Mississippi and Atchafalaya River estuaries in the United States, reveal similar patterns (Dinauer & Mucci, 2017; Lohrenz et al., 2018; Chen et al., 2020). Moreover, those locations are also characterized by huge salinity and temperature variations associated with anthropogenic environmental change (Casties et al., 2016; Pansch et al., 2018; Hinson et al., 2022; Kazanavičiūtė et al., 2024). On land, a similar pattern could be observed in impacted and protected sites between stressors such as temperature (i.e., urban ‘heat islands’) and pollutants (Flory & Borden, 2021).

Following a multiple-stressor framework with factorial stressor arrangements, our experiments demonstrated greater resistance to these stressors and higher phenotypic plasticity by the populations from the impacted habitats, particularly those more closely located to the city and experiencing the greatest disturbance connected to, for instance, the shipping industry. We demonstrate that populations inhabiting urban environments not only endure human-altered habitat conditions, but they are also more resistant to diverse stressors associated with global environmental change. Therefore, we speculate that those populations would be suitable for genetic rescue to increase population fitness through the introduction of new genetic material to endangered populations/species (Tallmon et al., 2004; Whiteley et al., 2015; Fitzpatrick et al., 2023). However, caution is needed in translocation of these populations, as they have a higher probability of establishing in non-native areas, especially other urban environments (Hufbauer et al., 2012; Borden & Flory, 2021; Perry & Göttert, 2024). Finally, additional studies testing resistance to various stressors of diverse taxa from multiple geographic locations and environments are needed to confirm the generality of our findings.

ACKNOWLEDGEMENT

EB was funded by the Alexander von Humboldt Sofja Kovalevskaja Award. JWED acknowledges financial support by the Alexander von Humboldt Foundation and RNC by the Alexander von Humboldt Foundation, as well as the Leverhulme Trust (ECF-2021-0001). We are grateful for valuable comments and suggestions from two reviewers and a handling editor.

REFERENCE

Alberti, M., Correa, C., Marzluff, J.M., Hendry, A.P., Palkovacs, E.P., Gotanda, K.M., et al. (2017) Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences*, 114(34), 8951-8956.

Alpert, S., Mielke, E., Burkhard, B. & Ismar, S. (2015) Neue Kartierung der Unterwasserhabitate im Naturschutzgebiet Schleimündung. *Seevögel*, 36(2), 10-14.

Alter, E.S., Tariq, L., Creed, J.K. & Megafu, E. (2020) Evolutionary responses of marine organisms to urbanized seascapes. *Evolutionary Applications*, 15;14(1), 210-232.

Ålund, M., Cenzer, M., Bierne, N., Boughman, J.W., Cerca, J., Comerford, M.S., et al. (2024) Anthropogenic change and the process of speciation. *Cold Spring Harbor Perspectives in Biology*, doi: 10.1101/cshperspect.a041455.

Aronson, M.F.J., La Sorte, F.A., Nilson, C.H., Katti, M., Goddard, M.A., Lepczyk, C.A., et al. (2014) A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proceedings of the Royal Society Biological Sciences*, 281, 20133330.

Borden, J.B. & Flory, L. (2021) Urban evolution of invasive species. *Frontiers in the Ecology and the Environment*, 19(3), 184-191.

Briski, E., Van Stappen, G., Bossier, P. & Sorgeloos, P. (2008) Laboratory production of early hatching *Artemia* sp. cysts by selection. *Aquaculture*, 282, 19–25.

Briski, E., Ghabooli, S., Bailey, S.A. & Macisaac, H.J. (2011) Assessing invasion risk across taxa and habitats: Life stage as a determinant of invasion success. *Diversity and Distributions*, 17, 593–602.

Briski, E., Bailey, S.A., Casas-Monroy, O., DiBacco, C., Kaczmarska, I., Lawrence, J.E., et al. (2013) Taxon- and vector-specific variation in species richness and abundance during the transport stage of biological invasions. *Limnology and Oceanography*, 58(4), 1361-1372.

Briski, E., Chan, F., MacIsaac, H.J. & Bailey, S.A. (2014) A conceptual model of community dynamics during the transport stage of the invasion process: a case study of ships' ballast. *Diversity and Distributions*, 20, 236-244.

Briski, E., Chan, F.T., Darling, J.A., Lauringson, V., MacIsaac, H.J., Zhan, A., et al. (2018) Beyond propagule pressure: importance of selection during the transport stage of biological invasions. *Frontiers in Ecology and the Environment*, 16(6), 345-353.

Briski, E., Kotronaki, S.G., Cuthbert, N.R., Bortolus, A., Campbell, M.L., Dick, J.T.A., et al. (2024) Does non-native diversity mirror Earth’s biodiversity? *Global Ecology and Biogeography*, 33, 48-62.

Bruno, J.F., Carr, L.A. & O’Connor, M.I. (2015) Exploring the role of temperature in the ocean through metabolic scaling. *Ecology*, 96, 3126-3140.

Buczkowski, G. (2010) Extreme life history plasticity and the evolution of invasive characteristics in a native ant. *Biological Invasions*, 12, 3343–49.

Cadotte, M.W., Yasui, S.L.E., Livingstone, S. & MacIvor, J.S. (2017) Are urban systems beneficial, detrimental, or indifferent for biological invasion? *Biological Invasions*, 19, 3489–3503.

Caizergues, A.E., Grégoire, A. & Charmantier, A. (2018) Urban versus forest ecotypes are not explained by divergent reproductive selection. *Proceedings of the Royal Society B: Biological Sciences*, 285(1882), 20180261.

Casties, I., Seebens, H. & Briski, E. (2016) Importance of geographic origin for invasion success: a case study of the North and Baltic Seas versus the Great Lakes-St. Lawrence River region. *Ecology and Evolution,* 6, 8318-8329.

Chen, B., Cai, W.-J., Brodeur, J.R., Hussain, N., Testa, J.M., Ni,W., et al. (2020) Seasonal and spatial variability in surface pCO_2 and air–water CO_2 flux in the Chesapeake Bay. *Limnology and Oceanography,* 65, 3046–3065

Cuthbert, R., Kotronaki, S.G., Dick, J.T.A. & Briski, E. (2020) Salinity tolerance and geographic origin mediate global alien amphipod invasions. Biology Letters, 16, 20200354.

Dinauer, A. & Mucci, A. (2017) Spatial variability in surface-water pCO_2 and gas exchange in the world's largest semi-enclosed estuarine system: St. Lawrence Estuary (Canada). *Biogeosciences*, 14, 3221–3237.

Dlugosch, K.M., Anderson, S.R., Braasch, J., Cang, F.A. & Gillette, H.D. (2015) The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology*, 24, 2095–111.

Dobbs, C., Nitschke, C. & Kendal, D. (2017) Assessing the drivers shaping global patterns of urban vegetation landscape structure. *Science of the Total Environment*, 592, 171–177.

Evens, D.H. (2008) Osmotic and ionic regulation. Cells and Animals. CRC Press, Boca Raton, 616 pp.

Fitzpatrick, S.W., Mittan-Moreau, C., Miller, M. & Judson, J.M. (2023) Genetic rescue remains underused for aiding recovery of federally listed vertebrates in the United States. *Journal of Heredity*, 114(4), July 2023, 354–366.

Gaitán-Espitia, J.D., Quintero-Galvis, J.F., Mesas, A. & D'Elía, G. (2016) Mitogenomics of southern hemisphere blue mussels (Bivalvia: Pteriomorphia): Insights into the evolutionary characteristics of the *Mytilus edulis* complex. *Scientific Reports*, 6, 26853.

Gerhardt, A., Bloor, M. & Mills, C.L. (2011). *Gammarus*: Important taxon in freshwater and marine changing environments. *International Journal of Zoology*, 2011, 2–4.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. (2001) Effects of size and temperature on metabolic rate. *Science*, 293, 2248-2251.

Giraudeau, M., Nolan, P.M., Black, C.E., Earl, S.R., Hasegawa, M. & McGraw, K.J. (2014) Song characteristics track bill morphology along a gradient of urbanization in house finches (*Haemorhous mexicanus*). *Frontiers in Zoology*, 11, 83.

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, M., Bai, X., et al. (2008) Global change and the ecology of cities. *Science*, 319, 756–60.

Hartig, F. (2020) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *R Package Version 0.3, 3.*

Haubrock, P.J., Balzani, P., Matsuzaki, S.I.S., Tarkan, A.S., Kourantidou, M. & Haase, P. (2021) Spatio-temporal niche plasticity of a freshwater invader as a harbinger of impact variability. *Science of the Total Environment*, 777, 145947.

Haufbauer, R.A., Facon, B., Ravigné, V., Turgeon, J., Foucaud, J., Lee, C.E., et al. (2012) Anthropogenically induced adaptation to invade (AIAI): contemporary adaptation to human-altered habitats within the native range can promote invasions. *Evolutionary Applications*, 5(1), 89–101.

Hawkins, S.J. (2012) Marine conservation in a rapidly changing world. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 22, 281-287.

Hinson, K.E., Friedrichs, M.A.M., St-Laurent, P., Da, F.& Najjar, R.G. (2022) Extent and Causes of Chesapeake Bay Warming. *Journal of the American Water Recources Association*, 58(6), 805-825.

Hulme, P.E. (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, 46, 10–18.

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Johnson, M.T.J. & Munshi-South, J. (2017) Evolution of life in urban environments. *Science*, 358, eaam8327.

Kaluza, P., Kölzsch, A., Gastner, M.T. & Blasius, B. (2010). The complex network of global cargo ship movement. *Journal of the Royal Society Interface*, 7, 1093-1103.

Kinnison, M.T. & Hendry, A.P. (2001) The pace of modern life II: From rates of contemporary microevolution to pattern and process. *Genetica*, 112-113, 145–164.

Kazanavičiūtė, E., Dickey, J.W.E., Soto, I., Haubrock, P.J., Kouba, A., Brennan, R.S., et al. (2024) Seasonal changes in biodiversity of native and non-native amphipod taxa under diverse environmental contexts. *Marine Biology*, 171, 156.

Knapp, S., Dinsmore, L., Fissore, L., Hobbie, S.E., Jakobsdottir, I., Kattge, J., et al. (2012) Phylogenetic and functional characteristics of household yard floras and their changes along an urbanization gradient. *Ecology*, 93(8), S83–S98.

Knöbel, L., Nascimento-Schulze, J.C., Sanders, T., Zeus, D., Hiebenthal, C., Barboza, F.R., et al. (2021) Salinity driven selection and local adaptation in Baltic Sea Mytilid mussels. *Frontiers in Marine Science*, 8, 692078.

Köhn, J. & Gosselck, F. (1989) Bestimmungsschlüssel der Malakostraken der Ostsee. Mitteilungen aus dem Museum für Naturkunde in Berlin. *Zoologische Reihe*, 65(1), 3-114.

Kosmidis, I. (2020) brglm2: Bias reduction in generalized linear models. *R Package Version 0.6,* 2, 635.

Lahr, E.C., Dunn, R.R. & Frank, S.D. (2018) Getting ahead of the curve: cities as surrogates for global change. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20180643.

Lenth, R. (2020). emmeans: Estimated marginal means, aka least-squares means (R package version 1.4. 8)[Computer software manual].

Linders, T.E.W., Schaffner, U., Eschen, R., Abebe, A., Choge, S.K., Nigatu, L., et al. (2019) Direct and indirect effects of invasive species: Biodiversity loss is a major mechanism by which an invasive tree affects ecosystem functioning. Journal of Ecology, 107, 2660-2672.

LLUR (Landesamt für Natur und Umwelt des Landes Schleswig-Holstein) (2001) Organozinnverbindungen in Hafensedimenten und biologische Effekte. Untersuchungen in Sedimenten und an der Strandschnecke (Littorina littorea L.) in schleswig-holsteinischen Küstengewässern. Flintbek: Landesamt für Natur und Umwelt (Landesamt für Natur und Umwelt des Landes Schleswig-Holstein: D, 20). Available at: https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url=https://umweltanwendungen.schleswig-holstein.de/Bestellsysteme/pdf/jahrbe01/Organozinnverbindungen.pdf&ved=2ahUKEwiPwb7BrfyJAxWCQvEDHbsKPDsQFnoECBwQAQ&usg=AOvVaw3rg-uAZ9zdLkgsZpzQ0xbY

Lockwood, J.L., Hoopes, M.F.& Marchetti, M.P. (2013) Invasion Ecology, Wiley-Blackwell A John Wiley & Sons, Ltd., Publication. 444 pp.

Lohrenz, S.E., Caib, W.-J., Chakrabortyc, S., Huangd, W.-J., Guoe, X., Hef, R., et al. (2018) Satellite estimation of coastal pCO2 and air-sea flux of carbon dioxide in the northern Gulf of Mexico. *Remote Sensing of Environment*, 207, 71–83.

Martin, R.A., Chick, L.D., Yilmaz, A.R. & Diamond, S.E. (2019) Evolution, not transgenerational plasticity, explains the divergence of acorn ant thermal tolerance across an urban–rural temperature cline. *Evolutionary Applications*, 12, 1678–87.

Meier, H.E.M., Dieterich, C., Gröger, M., Dutheil, C., Börgel, F., Safonova, K., et al. (2022) Oceanographic regional climate projections for the Baltic Sea until 2100. *Earth System Dynamics*, 13: 159–199.

McKinney, M.L. (2002) Urbanization, biodiversity and conservation. *Bioscience*, 52, 883–890.

McKinney, M.L. (2008) Effects of urbanization on species richness: A review of plants and animals. *Urban Ecosystems*, 11, 161–176.

Morón Lugo, S.C., Baumeister, M., Nour, O.M., Wolf F., Stumpp, M. & Pansch, C. (2020) Warming and temperature variability determine the performance of two invertebrate predators. *Scientific Reports*, 10, 6780.

Nikulina, A., Polovodova, I. & Schönfeld, J. (2008) Foraminiferal response to environmental changes in Kiel Fjord, SW Baltic Sea. *eEarth*, 3(1), 37–49.

Olden, J.D., Poff, N.L., Douglas, M.R., Douglas, M.E. & Fausch, K.D. (2004) Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology and Evolution*, 19, 18-24.

Pansch, C, Scotti, M, Barboza, F., Al-Janabi, B., Brakel, J., Briski, E., et al. (2018) Heat waves and their significance for a temperate benthic community: A near-natural experimental approach. *Global Change Biology*, 24, 4357–4367.

Perry, G. & Gottert, T. (2024) The City as an Evolutionary Hothouse—The Search for Rapid Evolution in Urban Settings. *Diversit*y, 16(6), 308.

Pörtner, H.-O. (2010) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, 213, 881-893.

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., et al. (2020) Scientists' warning on invasive alien species. *Biological Reviews*, 95(6), 1511–1534.

Rivera-Ingraham, G.A. & Lignot, J.-H. (2017) Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the questions for future research. *Journal of Experimental Biology*, 220, 1749-1760.

Ruppert, E.E., Fox, R.S. & Barnes, R.D. (2003) Invertebrate Zoology: A Functional Evolutionary Approach, Brooks/Cole Thomson Learning Inc., 7 Edition, 963 pp.

Santangelo, J.S., Rivkin, L.R. & Johnson, M.T. (2018) The evolution of city life. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20181529.

Sardain, A., Sardain, E. & Leung, B. (2019) Global forecasts of shipping traffic and biological invasions to 2050. *Nature Sustainability*, 2, 274-282.

Sol, D., Bartomeus, I., González-Lagos, C. & Pavoine, S. (2017) Urbanisation and the loss of phylogenetic diversity in birds. *Ecology Letters*, 20, 721–729.

Springer, S.A. & Crespi, B.J. (2007) Adaptive gamete-recognition divergence in a hybridizing *Mytilus* population. *Evolution*, 61(4), 772–783.

Tallmon, D.A., Luikart, G. & Waples, R.S. (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology and Evolution*, 19, 489–496.

Thomsen, J., Casties, I., Pansch, C., Körtzinger, A. & Melzner, F. (2012) Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. *Global Change Biology*, 19(4), 1017-1027.

Thompson, K.A., Rieseberg, L.H. & Schluter, D. (2018) Speciation and the city. *Trends in Ecology and Evolution*, 33, 815–26.

Todgham, A.E., Schulte, P.M. & Iwama, G.K. (2005) Cross-tolerance in the tidepool sculpin: the role of heat shock proteins. *Physiological and Biochemical Zoology*, 78(2), 133-144.

United Nations Statistics Division (2017). Population density and urbanization. United Nations. Available at: https://unstats.un.org/UNSD/Demographic/sconcerns/densurb/densurbmethods.htm.

van Kleunen, M., Essl, F., Pergl, J., Brundu, G., Carboni, M., Dullinger, S., et al. (2018) The changing role of ornamental horticulture in alien plant invasions. *Biological Reviews*, 93, 1421–37.

Verein Jordsand zum Schutze der Seevögel und der Natur e.V. (2019) Accessed on 28.9.2019. Available at: https://www.jordsand.de

Westneat, D.F., Potts, L.J., Sasser, K.L. & Shaffer, J.D. (2019) Causes and consequences of phenotypic plasticity in complex environments. *Trends in Ecology and Evolution*, 34, 555–68.

Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C. & Tallmon, D.A. (2015) Genetic rescue to the rescue. *Trends in Ecology and Evolution*, 30, 42–49.

Winchell, K.M., Reynolds, R.G., Prado-Irwin, S.R., Puente-Rolon, A.R. & Revell, L.J. (2016) Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution*, 70, 1009–22.

Zettler, M.L. & Zettler, A. (2017) Marine and Freshwater Amphipoda from the Baltic Sea and Adjacent Territories. Harxheim, Germany: ConchBooks (Die Tierwelt Deutschlands und der angrenzenden Meeresteile, 83. Teil).

Table 1. Outline of experimental parameters used for a) temperature - pCO$_2$ *Mytilus* sp., *G. locusta*, and *G. salinus*, b) temperature-salinity - *Mytilus* sp., and c) temperature-salinity - *G. locusta* and *G. salinus* experiments.

a) Temperature – pCO$_2$ experiments – *Mytilus* sp., *G. locusta*, and *G. salinus*

Treatment	Ambient	T1	T2	T3	T4	T5	T6	T7
Temperature (°C)	16	16	16	16	24	24	24	24
pCO$_2$ (μatm)	400	1 600	2 700	3 500	400	1 600	2 700	3 500

b) Temperature – salinity experiments – *Mytilus* sp.

Treatment	Ambient	T1	T2	T3	T4	T5
Temperature (°C)	16	20	25	16	20	25
Salinity (g/kg)	16	16	16	27	27	27

c) Temperature – salinity experiments – *G. locusta* and *G. salinus*

Treatment	Ambient	T1	T2	T3	T4	T5
Temperature (°C)	17	20	24	17	20	24
Salinity (g/kg)	14	14	14	25	25	25

Figure legends:

Figure 1. Sampling locations used in the study, where individuals of species populations were collected. Schleimünde served as the protected site, whereas Falckenstein beach, Kiel canal, and downtown Kiel served as human-altered sites.

Figure 2. Percentage mortality for *Mytilus* sp. from protected and human-altered habitats for the temperature-pCO_2 experiment (left) and the temperature-salinity experiment (right).

Figure 3. Percentage mortality for *Gammarus locusta* from protected and human-altered habitats for the temperature-pCO_2 experiment (left) and the temperature-salinity experiment (right).

Figure 4. Percentage mortality for *Gammarus salinus* from protected and human-altered habitats for the temperature-pCO_2 experiment (left) and the temperature-salinity experiment (right).