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Marine Uguen[1,*], Sylvie M Gaudron[1,2], Katy R Nicastro[1,3,4], Gerardo I Zardi[3,5], Nicolas Spilmont[1], Solène Henry[1], Laurent Seuront[1,3,6]

(1) Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, IRD, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, Station marine de Wimereux, F-59000 Lille, France.
(2) Sorbonne Université, UFR 927, F-75005 Paris, France.
(3) Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa
(4) CCMAR–Centro de Ciencias do Mar, CIMAR Laboratório Associado, Universidade do Algarve, Campus de Gambelas, Faro, 8005-139 Portugal
(5) Laboratoire de Biologie des Organismes et Ecosystèmes Aquatiques (BOREA), Université de Caen-Normandie, MNHN, SU, UA, CNRS, IRD, Esplanade de la paix, 14032 Caen Cedex, France
(6) Department of Marine Resources and Energy, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo, 108-8477 Japan

* Corresponding author: marine.uguen@univ-lille.fr

ORCID IDs
MU: 0000-0001-5547-608X
SMG: 0000-0001-6189-3824
KRN: 0000-0002-7071-141X
GIZ: 0000-0001-8798-5794
NS: 0000-0002-2380-1113
SH: 0000-0002-6309-7159
LS:0000-0002-0051-5202

Abstract

Plastic pollution and ongoing climatic changes exert considerable pressure on coastal ecosystems. Unravelling the combined effects of these two threats is essential to management and conservation actions to reduce the overall environmental risks. We assessed the capacity of a coastal ecosystem engineer, the blue mussel *Mytilus edulis*, to cope with various levels of aerial heat stress (20, 25, 30 and 35°C) after an exposure to substances leached from beached and virgin low-density polyethylene pellets. Our results revealed a significant interaction between temperature and plastic leachates on mussel survival rates. Specifically, microplastic leachates had no effect on mussel survival at 20, 25 and 30°C. In turn, mussel survival rates significantly decreased at 35°C, and this decrease was even more significant following an exposure to leachates from beached pellets; these pellets had higher concentration of additives

compared to the virgin ones, potentially causing a bioenergetic imbalance. Our results stress the importance of adopting integrated approaches combining the effects of multiple environmental threats on key marine species to understand and mitigate their potential synergistic effects on ecosystem dynamics and resilience in the face of the changing environment.

Keywords

warm, global change, contaminant, emersion, keystone species

1. Introduction

Plastics and their associated chemicals have become a global concern from both scientific and societal perspectives. The ever-increasing plastic production and poor waste management has led to a permanent contamination of the biosphere by plastic items [1–3]. Coastal systems are some of the most heavily plastic-polluted ecosystems [4,5]. Plastics may pose a serious threat to organisms not only through physical damages [6,7], but also through the far less studied consequences of their chemical content [8,9]. Plastic debris may noticeably act as vectors for numerous chemical compounds that can be hazardous depending on the nature of the chemicals and their concentrations [10,11], which can vary during plastic lifetime. Virgin plastics are essentially composed of plastic polymers to which a range of additives are incorporated during their manufacture in order to improve their performances [12], with *e.g.* phthalates making up 10 to 60% of the PVC weight [13]. Then, when released in the environment, a range of environmental contaminants, potentially more toxic than additives (*e.g.* heavy metals, pesticides), may be adsorbed on plastic particle surface and accumulate [14,15], at concentrations up to 6 orders of magnitude higher than in the environment [16] and then be desorbed [8]. These sorption/desorption phenomena noticeably increases as particle size decreases [17]. As such, plastics in general and microplastics in particular (*i.e.* particles smaller

than 5 mm) may act both as a sink and a source of contaminants, exposing organisms to complex and potentially toxic cocktails of chemical compounds [8,9,18].

As climate change progresses, the effects of extreme weather conditions, including the increased frequency, intensity, and duration of heat stress, are expected to become more pronounced [19–21]. In intertidal ecosystems, these events are particularly stressful, *e.g.* the body temperature variation experienced by mussel specimens between emersion and immersion can exceed 20°C (*e.g.* [22,23]), and can cause damages and mass mortality episodes, especially in marine invertebrates such as mussels [22,24–26]. Noticeably, the temperature tolerance of organisms is dependent on their health status, which can further be compromised by the presence of contaminants in seawater [27–29]. Even though plastic pollution and heat stress *de facto* frequently coincide, only a handful of studies have explored their combined repercussions. These essentially include adverse effects on survival, physiological processes, and cellular functions [30–33] though exceptions exist [34–36]. Despite the plethora of studies that assessed the effects of microplastic leachates on various aspects of the biology and ecology of marine organisms [8], to the best of our knowledge the combined impact of plastic leachates and heat stress has not been addressed. Given the potentially high impact of these two stressors on intertidal ecosystems, the objective of this study was to assess the impact of microplastic leachates on the ability of a key intertidal ecosystem engineer [37], the blue mussel *Mytilus edulis*, to survive to aerial heat stress. Leachate solutions were consistently prepared from low-density polyethylene pellets that were either virgin (*i.e.* raw commercially available pellets) or found stranded on the beach, and aerial heat stress was simulated at 4 different temperatures (*i.e.* 20, 25, 30 or 35°C). We hypothesised that plastic leachate exposure alters the mussel ability to withstand an aerial heat stress event and that leachates from beached pellets will have a more deleterious impact than virgin ones because they have the potential to accumulate additional toxicants when dispersed in the environment.

2. **Material and Methods**

a) Study organisms

A total of 720 specimens of the blue mussel *Mytilus edulis* (3 to 4 cm in shell length) were collected in September 2022 from the intertidal rocky shore at Pointe aux Oies (Wimereux, France; 50°47’08.3"N, 1°36’03.9"E) along the French coast of the eastern English Channel. Prior to the experiments, mussels were acclimated in the laboratory for 24 h in 85 L tanks filled with oxygen saturated natural seawater [38] representative of *in situ* conditions (T = 20°C; S = 33).

b) Microplastics and leachate solution preparation

Microplastic leachate solutions (MPL) were prepared from either (i) commercially available (Materialix Ltd., London, United Kingdom) low-density spheroidal polyethylene pellets (with typical longest and shortest axes respectively 4.14 ± 0.21 mm and 1.89 ± 0.10 mm in length; V-MPL treatment) or (ii) cylindrical beached pellets (typically 4.04 ± 0.56 mm in width and 3.03 ± 0.82 mm in height; B-MPL treatment) collected from the high-tide mark sediment surface of the nearby beach (Ambleteuse, France; 50°80'61.9"N 1°60'31.34"E). Beached pellets were consistently identified as low-density polyethylene using Fourier transform infrared (FTIR) analysis with an Aldrich FTIR database search set at 97.5% correlation setting. GC-HRMS analyses showed that virgin pellets were composed of 5 additives, including 4 plasticisers and 1 antioxidant. In contrast, beached pellets contained 7 additives, 4 plasticisers and 3 antioxidants and in a quantity typically 2 to 4-fold higher than in virgin pellets. For more details about the polymer and chemical content identification and the method used, see Supplementary Material, S1.

Each type of pellets was mixed with natural aerated seawater for 24h [38–41] at 20°C at a

concentration of 10 g of pellets per litre (*ca*. 400 pellets per litre, or equivalently 20 mL of pellets per litre; [39]). After 24 h, the experiment was performed using the solution alone (*i.e.* without any pellets). A control solution (Control) was prepared by incubating aerated seawater for 24 h at 20°C. Consistent oxygen saturation and seawater renewal were ensured for each treatment during every immersion cycle.

c) Experimental design

The experiment was designed to mimic the temperature experienced by *M. edulis* during a typical tidal cycle at the sampling site (*i.e.* 6 h immersion/emersion with a sea surface and air temperature of 20°C; see SOMLIT 2022 data for Wimereux in September; https://www.somlit.fr/visualisation-des-donnees/), with a one-off emersion heat stress event representative of observed air temperature at the study site during the summer 2022 (25, 30 and 35°C; air temperature record = 39.6°C [42]). A previous study conducted at our study site, using robomussels, revealed that mussel body temperatures occasionally exceeded 35°C, reaching a maximum recorded temperature of 41.7°C, while the seawater temperature remained around 15°C, resulting in a delta temperature of over 20°C [22].

Specifically, the 72-hour experiment consisted of 6 successive immersion-emersion cycles that were conducted (i) under naturally occurring seawater and air temperatures at our study site in September (*i.e.* 20°C) as a control, and (ii) under a 6-hour aerial heat stress event of 25, 30 or 35°C after one immersion-emersion-immersion cycle at 20°C and followed by 4 immersion-emersion recovery events at 20°C (Fig. 1).

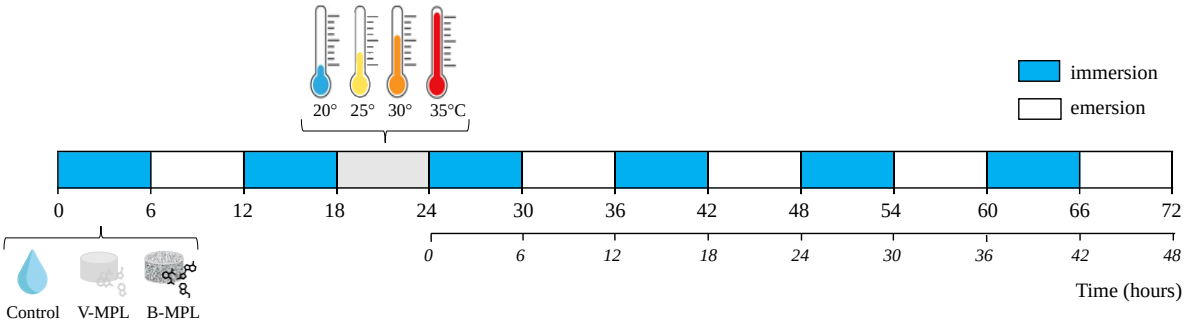

Figure 1: Schematic diagram of the immersion-emersion experiments. Mussels were immerged (blue rectangles) during 6 hours in Control, Virgin microplastic leachate (V-MPL) or Beached microplastic leachate (B-MPL) solutions and emerged (white rectangles) during 6 hours consistently at an aerial temperature of 20°C (control temperature) except during the second emersion (grey rectangle) where the mussels were exposed to a low (25°C), moderate (30°C) or high (35°C) aerial heat stress event. The numbers indicate the time in hours after the beginning of the microplastic leachate stress (normal font) or after the thermal stress (italic font).

For each experimental treatment (Temperature × Solution), 20 mussels were used in triplicates (*i.e.* N = 60 mussels for each of the 12 experimental trials). During the immersion phase, the mussels were incubated in a 1.5 L glass jar containing 1 L of Control, V-MPL, or B-MPL solutions. Immersions lasted for 6 hours at a temperature of 20 °C (Fig. 1). The emersion consisted in placing the mussels on natural seawater saturated paper towels in sealed 1.5 L glass jars to maintain around 95% relative humidity to avoid desiccation or evaporation [22]. For the aerial heat stress, the jars were placed in incubators (MIR-154, Panasonic, Japan; temperature resolution ± 0.3 °C) heated either at 20, 25, 30 or 35°C for 6 h (Fig. 1). Relative humidity and air temperature experienced by mussels inside the jars were monitored using Hygro Buttons 23 (Proges-Plus, resolution 0.5°C and 1%) at 5 min interval (Supplementary Materials, S2).

d) Endpoint and Statistical analyses

After each immersion and emersion, the status of each mussel was visually checked. Opened mussels that did not respond to foot probing, *i.e.* no valve closure, were discarded and recorded

as dead [22,43–45].

To study the effects of the factors 'Temperature', 'Solution' and their interaction 'Temperature × Solution', data were analysed using a 2-way ANOVA with Solution (Control, V-MPL, B-MPL) and Temperature (20°C, 25°C, 30°C, 35°C) as fixed factors and percentage of survived mussels at the end of the experiment as the dependent factor. Significant effects were examined using Tukey-HSD post-hoc test. The data met the assumption required for the ANOVAs; homogeneity of variances and dispersion of the residuals were checked using the package 'DHARMa' (see Supplementary Materials, S3; [46]).

Lethal times at 50% of mortality (LT50; in hours) and their lower and upper fiducial confidence limits (CL) for each solutions at 35°C — the only temperature where a significant mortality was recorded — were estimated using a binomial generalised linear model with a probit link function using the package 'ecotox' [47–50]. The data met the assumption required for the generalised linear model; homogeneity of variances and dispersion of the residuals were checked using the package 'DHARMa' (see Supplementary Materials, S4; [46]). To determine the existence of differences between the LT50 at 35°C of each treatment, a ratio test was used [49]. Exact *p* values are given in Supplementary Materials S5, S6 and S8. All statistical analyses were performed using the R Core Team software (2022).

3. Results

a) Survival rates

Survival rates (mean ± SD) significantly decreased only after an aerial heat stress exposure at 35°C for all solutions, *i.e.* Control (48.3 ± 14.4%), V-MPL (53.3 ± 7.6 %) and B-MPL (26.7 ± 7.6%); Temperature × Solution, $p < 0.01$; Tukey test*:* $p < 0.001$; S5, S6; Fig. 2). Specifically, at 35°C, significantly more mussels —5 on average— died when exposed to B-MPL compared to those exposed to V-MPL and Control seawater (Temperature × Solution, $p < 0.01$; S6, Tukey

test: $p < 0.01$ and $p < 0.05$, respectively; Fig. 2). No significant differences in mussel mortality were found between V-MPL and Control seawater treatments at any of the aerial heat stress temperature tested (Temperature × Solution, $p < 0.01$; S6; Tukey's test: $p > 0.05$; Fig. 2).

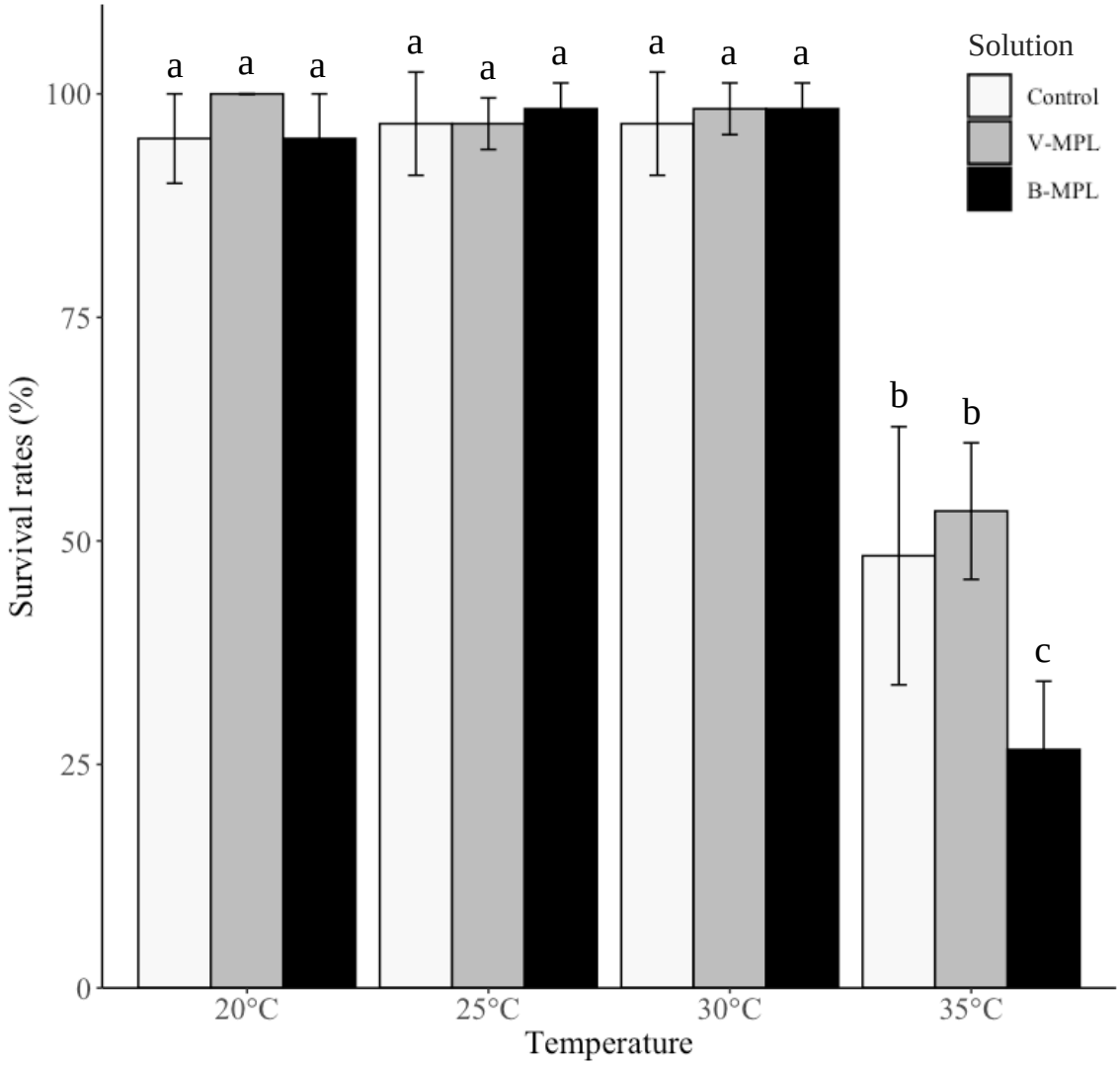

Figure 2: Survival rates (%; mean ± Standard Deviation) of *Mytilus edulis* at the end of the 72 h experiment after an exposure to control seawater (light grey), virgin (grey) and beached (black) microplastic leachate solutions combined with an aerial heat stress event at different temperatures. Letters depict significant differences among Temperature × Solution treatments ($p < 0.05$; Two-way ANOVA, Tukey post-hoc comparison). For details about the exact *p-values*, please refer to the Supplementary Materials S5, S6.

b) Lethal time after a 35°C aerial heat stress

When exposed to a 35°C aerial heat stress, the LT50 (CL) of mussels exposed to control seawater, *i.e.* 69.5 h (64.4 – 78.0 h), was not significantly different from the LT50 recorded following exposure to V-MPL, *i.e.* 71.1 h (67.3 – 77.0 h; ratio test: $p > 0.05$, S7, S8). In contrast, mussels exposed to B-MPL were less resistant to a 35°C aerial heat stress, as LT50 was reached in significantly less time, *i.e.* 59.4 h (57.2 – 62.0 h), than the other two treatments (ratio test: $p < 0.001$, S7, S8).

4. Discussion

We provide the first evidence of the combined effect of temperature and plastic leachates on the survival rate of the keystone ecosystem engineer, *Mytilus edulis*. At temperatures ranging from 20 to 30°C, the presence of leachates, either from virgin or beached microplastics, did not affect the mortality rates of *M. edulis*. In sharp contrast, under conditions of high thermal stress (35°C), and in agreement with previous studies [22,51], there was a significant decline in survival rates for all treatments, especially when mussels were exposed to leachates from beached microplastics. These results raise concern, given that thermal events with mussel body temperature reaching more than 35°C during summer emersion at the study site occurred *ca.* 11 times over their 84-day time series [22], a scenario expected to intensify under future climate projections [20]. Sub-lethal synergistic effect of temperature and contaminant, *e.g.* [52–56], including plastics, *e.g.* [31,57–59], have previously been documented in mussels under conditions of immersion. This present work, however, provides the first evidence of an enhanced thermal mortality through an exposure to microplastic leachates.

The adverse synergistic effect observed here is likely due to a bioenergetic imbalance, *i.e.* the energy costs exceed the available energy supply. Leachates originating from various plastics such as car tire rubber, polypropylene, polyethylene terephthalate, polystyrene, polyvinyl chloride, and noticeably even bioplastics are capable of harming mussel cells by interfering with processes like lysosomal function, neurotransmission, oxidative stress and antioxidant

defences; see [60,61] for details on leachates composition. Under low and moderate thermal stress conditions, the detoxification and repair cost may be low enough to supply basal maintenance [29,62]. In turn, at high temperatures, the mussel heat shock response cost and cell damages caused by aerial heat stress [63] are added to those of plastic leachates. In this context, our results suggest that the processes used to counteract the cellular damage caused by these combined stressors may become too costly and create a bioenergetic deficit, leading to high mortality [29,62].

Noticeably, *M. edulis* survival rates following a 35°C aerial heat stress varied depending on the pellet's history, with beached microplastic leachates (from pellets collected in the environment and used at a locally realistic concentration [64]) being significantly more harmful than virgin microplastic leachates (from commercially available pellets). The more severe effect of B-MPL compared to V-MPL has previously been shown in *Perna perna* mussel embryo [41] and also in a wide range of other species, *e.g.* in sea urchins (*Paracentrotus lividus* [65,66]), jellyfish [65], gastropods [64] and even in dune plants [67], although exceptions exist such as in zebrafish [65], copepods and another sea urchin (*Lytechinus variegatus* [68,69]).

The strongest effect observed for beached microplastic leachates compared to virgin ones after the high aerial heat stress is likely related to the pellet chemical content. Indeed, virgin pellets contained less additives, which were 2- to 4-fold less concentrated than in beached ones. The additives characterised were mainly phthalate plasticisers (*i.e.* Dimethyl phthalate, DMP; Diethyl phthalate, DEP; Di-n-butyl phthalate, DBP and Diisobutyl phthalate, DIBP) and Bisphenol S antioxidant, which are known to have various detrimental effects on marine invertebrates, for reviews see [70–72]. In addition, due to their permanence in the natural environment, beached pellets are also likely to be loaded with a harmful cocktail of environmental contaminants such as Persistent Organic Pollutants and heavy metals [73,74]. This is in sharp contrast with virgin pellets which are essentially composed of functional

additives (*e.g.* flame retardants, plasticisers, colorants [75]). Although mussel survival rates were noticeably not affected at low temperatures when combined with either type of plastic leachates, this does not rule out the existence of sublethal effects such as behaviour, growth and reproduction which are likely to have longer term detrimental effects on the biology, ecology and ultimately survival [62]. The resolution of these specific issues lies beyond the scope of the present work, but warrants the need for further studies.

5. Conclusion

Thermal stress and plastic pollution are prominent challenges in the Anthropocene. In the context of the anticipated rise in frequency, severity, and duration of extreme heat events [20], coupled with the growing production of plastic and the subsequent accumulation of plastic waste in the environment [76], our results raise concerns and highlight the critical need for multi-stress studies to fully comprehend the impacts of these stressors on organisms. In turn, increase in temperature has been shown to enhance the leaching of plastic associated chemical compounds [77–79], and hence their potential toxicity, highlighting the need for further studies to understand the fate of plastic-associated chemicals in warming ecosystems. Considering the engineering role of the species used in this study, the combined impact of environmentally realistic temperature [22], as well as the type and concentration of plastic pellet [64], could pose a significant threat not only to the species itself but also to the overall ecosystem that relies on these species for sustenance.

Ethics

Under French regulations, the present work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility

The data and scripts that support the findings of this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.gtht76hst.

Supplementary material is available online.

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.

Author contributions

Marine Uguen: Conceptualisation, Methodology, Investigation, Data curation, Formal Analysis, Writing - Original Draft, Editing. **Sylvie M Gaudron**: Supervision, Resources, Methodology, Investigation, Writing ‑ Review & Editing. **Katy R Nicastro**: Supervision, Methodology, Investigation, Writing ‑ Review & Editing**. Gerardo I Zardi**: Methodology, Investigation, Writing ‑ Review & Editing. **Nicolas Spilmont:** Methodology, Investigation, Writing ‑ Review & Editing. **Solène Henry:** Investigation, Writing – Review & Editing. **Laurent Seuront:** Supervision, Resources, Methodology, Investigation, Writing ‑ Review & Editing, Funding acquisition.

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