## **Global warming**

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**Abstract:** The ecological risk assessment of microplastics under global warming receives increasing attention. Yet, such studies mostly focused on increased mean temperatures (MT), ignoring another key component of global warming, namely daily temperature fluctuations (DTF). Moreover, we know next to nothing about the combined effects of multigenerational exposure to microplastics and warming. In this study, Daphnia magna was exposed to an environmentally relevant concentration of polystyrene microplastics (5  $\mu$ g L-1) under six thermal conditions (MT: 20°C, 24°C; DTF: 0°C, 5°C, 10°C) over two generations to investigate the interactive effects of microplastics and global warming. Results showed that microplastics had no effects on Daphnia at standard thermal conditions (constant 20°C).

**Keywords:** Energy metabolism, Environmentally relevant concentrations, Global warming, Heat tolerance, Hormesis, Microplastics

The ecological risk assessment of microplastics has attracted great attention as they are ubiquitous and hard to degrade in natural environments, and thought to have negative effects on organisms (Guilhermino et al., 2021). Most studies, however, exposed organisms to unrealistically high concentrations of microplastics, potentially overestimating the effects of microplastics at environmentally relevant concentrations. The few studies exploring the exposure to microplastics at environmentally relevant concentrations mainly showed little or no effects on organisms (Guven et al., 2018, Revel et al., 2020). Yet, recent studies demonstrated that multigenerational long-term exposure to microplastics can cause stronger biological effects even at low concentrations (Liu et al., 2020, Chang et al., 2022). For instance, two generations of exposure to polystyrene (5  $\mu$ g L-1) decreased the heartbeat rate of D. magna (Chang et al., 2022), and three generations of exposure to polystyrene (1  $\mu$ g L-1) increased the growth rate and reproduction of D. pulex (Liu et al., 2020). Given their long persistence in natural systems, multigenerational exposure to environmentally relevant concentrations is therefore essential for assessing the real effects of microplastics.

The effects of toxicants are influenced by other environmental stressors, of which temperature is a critical one to consider given our warming planet (Noyes et al., 2009, Dinh et al., 2023). Indeed, the toxicity of many contaminants is higher under warming, which is known as the "climate-induced toxicant sensitivity" (CITS) concept (Noyes and Lema, 2015). Most studies on the effects of warming on the toxicity of contaminants focused on traditional contaminants (e.g. pesticides: Verheyen et al.,

2019a; metals: Zhang et al., 2018), whereas only recently attention went to emerging contaminants such as microplastics. The few studies on this typically investigated the effect of a higher mean temperature (MT) on microplastics' toxicity and resulted in mixed findings (Jaikumar et al., 2018, Sadler et al., 2019, Lyu et al., 2021). Sadler et al. (2019) found that an elevated temperature (18°C vs 24°C) did not affect the toxicity of microplastics, while Jaikumar et al. (2018) reported that the acute sensitivity of Daphnia to microplastics increased sharply with temperature (18°C, 22°C and 26°C), and similarly Lyu et al. (2021) reported that the toxicity of microplastics in Daphnia was more prominent at higher mean temperatures (15°C, 20°C and 30°C). While these studies provided valuable insights, they did not consider another key factor of global warming: the increase in daily temperature fluctuations (DTF). The increase in DTF may be more harmful than the increase in MT, moreover the effect of DTF may be larger at a high MT because more extreme temperatures, and associated costs, will be experienced during a DTF cycle (Colinet et al., 2015). However, we currently know next to nothing about how DTF together with an increase in MT affects the toxicity of microplastics (but see Chang et al., 2022). Note that nearly all tests on the toxicity of microplastics used constant temperatures, thereby ignoring the widespread DTF in current thermal regimes (Colinet et al., 2015). Therefore, including DTF is of crucial importance to increase realism in ecological risk assessment of microplastics.

The interplay between ecotoxicology and global change biology can go in both directions. Warming cannot only alter the toxicity of toxicants, toxicants can also affect the sensitivity of organisms to warming that is known as the "toxicant-induced climate change sensitivity" (TICS) (Noyes et al., 2009, Verheyen et al., 2019a). Previous studies demonstrated that traditional toxicants in general decreased the heat tolerance of aquatic organisms (e.g. Patra et al., 2007; Noyes et al., 2009; Verheyen et al., 2019a). For instance, the critical thermal maximum (CTmax, the high temperature whereby animals lose locomotor abilities) was reduced by exposure to the metals cadmium and chromium in marine prawn Macrobrachium rosenbergii (Rosas, 1993), and by exposure to the pesticides endosulfan and chlorpyrifos in different freshwater fish species (Patra et al., 2007). Evaluating the effects of toxicants on the heat tolerance of organisms is crucial to understand the fate of natural populations under global warming in the presence of toxicants (Verheyen et al., 2019a). Yet, whether and how emerging contaminants like microplastics affect the heat tolerance of organisms remains unknown.

There is increasing attention for energy budgets in ecotoxicology as these may explain effects on life history traits (Verheyen and Stoks, 2020) and are thought to be the underlying drivers of interactions between stressors (Sokolova, 2013). Stressors such as toxicants and warming may indeed negatively affect the balance between energy gains and costs, thereby impairing the net energy budget. They may reduce

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energy availability (e.g. because of reduced feeding), as well as increase energy consumption (e.g. because of costly defense mechanisms) (Sokolova, 2013). For example, it was shown that the negative effect on the net energy budget contributed to increased toxicity of the pesticide chlorpyrifos under warming (Verheyen and Stoks, 2020). Effects of microplastics on energy budgets are still not fully understood.

## References

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