Environmental Pollution 363 (2024) 125109

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Synergistic interaction between a toxicant and food stress is further exacerbated by temperature☆

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ARTICLE INFO

Keywords:
Pesticide-effects
Effect-models
Synergism
Environmental stressors
Combined stress

ABSTRACT

Global biodiversity is declining at an unprecedented rate in response to multiple environmental stressors. Effective biodiversity management requires deeper understanding of the relevant mechanisms behind such ecological impacts. A key challenge is understanding synergistic interactions between multiple stressors and predicting their combined effects. Here we used *Daphnia magna* to investigate the interaction between a pyrethroid insecticide esfenvalerate and two non-chemical environmental stressors: elevated temperature and food limitation. We hypothesized that the stressors with different modes of action can act synergistically. Our findings showed additive effects of food limitation and elevated temperature (25 °C, null model effect addition (EA)) with model deviation ratio (MDR) ranging from 0.7 to 0.9. In contrast, we observed strong synergistic interactions between esfenvalerate and food limitation at 20 °C, considerably further amplified at 25 °C. Additionally, for all stress combinations, the synergism intensified over time indicating the latent effects of the pesticide. Consequently, multiple stress substantially reduced the lethal concentration of esfenvalerate by a factor of 19 for the LC_{50} (0.45–0.024 μg/L) and 130 for the LC_{10} (0.096–0.00074 μg/L). The stress addition model (SAM) predicted increasing synergistic interactions among stressors with increasing total stress.

1. Introduction

Maintaining biodiversity of surface water ecosystems is paramount for providing essential ecosystem services. However, these ecosystems are increasingly subjected to multiple anthropogenic stressors that compromise their ecological status. Large-scale studies have revealed that a staggering 90% of European lowland rivers are affected by multiple stressors (Schinegger et al., 2012), with approximately 60% of water bodies failing to achieve the "good ecological status" stipulated by the European Water Framework Directive (WFD) (EEA, 2018). Despite this widespread degradation, a key challenge remains in understanding the synergistic interactions between chemical and non-chemical stressors, which are often more harmful in combination than acting alone. This knowledge gap is particularly crucial in the field of multiple stressor ecology, where the combined impacts of climate change, chemical pollution, and other physical stressors are reshaping ecosystems by altering the composition of natural communities and affecting ecosystem services (Chara-Serna et al., 2019; Gerner et al., 2017; Liess et al., 2021; Siddique et al., 2024). Our study addresses this gap by investigating how these stressors interact, particularly focusing on their synergistic effects and the potential implications for improving biodiversity management and conservation strategies.

Investigating the cumulative risks from multiple chemical and non-chemical stressors is receiving increased attention. For instance, Schäfer et al. (2016) elucidated that over 85% of analyzed river sites in Germany were at risk from at least three stressors, corroborating the findings of Schinegger et al. (2012) regarding European waters. Notably, European riverine ecosystems seem to be more susceptible to synergistic effects of multiple stressors than lakes, where nutrient enrichment stands out as a dominant stressor (Birk et al., 2020). The growing recognition of the critical role of multiple stressors has spurred research initiatives to understand and mitigate their effects on aquatic ecosystems (Birk et al., 2020; Hering et al., 2015; Navarro-Ortega et al., 2015).

Global warming and pollutants are two major stressors contributing

☆ This paper has been recommended for acceptance by Philip N. Smith.

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https://doi.org/10.1016/j.envpol.2024.125109
Received 12 June 2024; Received in revised form 16 September 2024; Accepted 9 October 2024
Available online 11 October 2024

to biodiversity loss, particularly in aquatic ecosystems (Bernhardt et al., 2017; Dinh et al., 2022; Jaureguiberry et al., 2022; Ortiz et al., 2021; Sylvester et al., 2023). Elevated temperature has been shown to exacerbate the toxicity of numerous toxicants (Polazzo et al., 2022; Shahid et al., 2024; Siddique et al., 2021; Verheyen et al., 2022), suggesting that global warming is likely to intensify the effects of pesticide pollution (Kattwinkel et al., 2011; Polazzo et al., 2022). Although temperature plays a crucial role in the physiology and performance of ectotherms, heat waves can cause acute physiological stress (Sokolova and Lannig, 2008; Vannote and Sweeney, 1980) by increasing metabolic demands required for thermal tolerance (Cherkasov et al., 2006; Madeira et al., 2018).

Since organisms in the field regularly face periods of low food availability, effects become even more prominent under multiple stress conditions (Adamo et al., 2012). Some investigations suggest that the detrimental impacts of heat waves might only become evident or be magnified under limited food resources (Adamo et al., 2012; Lee and Roh, 2010). Even though multiple stress interactions are common (Holmstrup et al., 2010; Laskowski et al., 2010), they are often overlooked in ecological risk assessment. Studies investigating the interactive effects of agricultural toxicants with elevated temperature or food shortage are particularly scarce. Experiments that incorporate all these three stressors in combination are even rarer. However, there is increasing awareness about the cumulative and potentially delayed impacts of these stressors (Arambourou and Stoks, 2015; Janssens et al., 2017; Macaulay et al., 2021b; Siddique et al., 2021). They typically impose transient stress over time that animals often encounter simultaneously or successively (Bowler et al., 2020; Liess et al., 2016; Rillig et al., 2023). For efficient ecosystem management, it's crucial to comprehend both the interactive and delayed impacts of multiple stressors, allowing us to predict the cumulative effects. In fact, predicting the combined effects of complex multiple stressors remains one of the pressing challenges in ecotoxicology (Cote et al., 2016; Jones, 1975; Liess et al., 2001; Liess et al., 2016; Schafer and Piggott, 2018).

Effects of multiple stressors are generally evaluated in relation to the corresponding null models. For predicting the combined effects of non-interacting multiple stressors, Concentration Addition (CA) is generally applicable to predict the effects of stressors with similar modes of action (Bliss (1939)), while Effect Addition (EA) is considered for stressors with different modes of action Loewe and Muischnek (1926)). However, in the case of interacting multiple stressors, combined effects often deviate from these null models, requiring advanced approaches for reliable predictions (Liess et al., 2016; Thompson et al., 2018). In comparison to CA and EA, the Stress Addition Model (SAM) was developed to predict the combined effects of interacting stressors, such as chemical and environmental stressors (Liess et al., 2016). The prediction of the SAM is based on three key assumptions: (i) each individual has a specific stress tolerance capacity against stress, (ii) all stressors can be converted into a general stress level from 0 (no mortality) to 1 (100% mortality) using stress-related mortality as a common metric, and (iii) the combined effect is estimated by adding up the general stress levels induced by different independent stressors.

Accordingly, we aimed to quantify the combined effects of the pyrethroid esfenvalerate in combination with food limitation and warming – key environmental stressors under climate change. We hypothesized that (i) the survival of daphnids exposed to esfenvalerate would be significantly increased by additional stressors with different modes of action, (ii) and the combined effects of multiple stressors would exceed those predicted conventional models to identify possible interactions. We also sought to determine whether the stress addition model (SAM; Liess et al. (2016)), which can quantify synergism between chemical and non-chemical stressors, can better predict the combined effects.

2. Materials and methods

2.1. Experiment design

We used 24 h old neonates of *D. magna* sourced from the clone "Aachen 5" and cultured at the Department of Ecotoxicology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany. The culture was maintained in Aachener Daphnien Medium (ADaM) (Klüttgen et al., 1994) at a constant temperature of 20.0 ± 1 °C, and under a 16/8-h light/dark cycle to facilitate continuous amictic reproduction (Sebens, 1982). Daphnids were fed with green algae "*Desmodesmus subspicatus*", and yeast as described earlier (Shahid et al., 2019).

We investigated the combined effects of a common pyrethroid insecticide esfenvalerate, and two environmental stressors: food limitation and elevated temperature. Since the *Daphnia* culture was maintained at 20 °C, we considered it as a reference temperature. An increase to 25 °C brings it closer to the upper threshold of their thermal tolerance, representing a meaningful thermal stress that could exacerbate the effects of other stressors like chemicals. Therefore, we selected 25 °C as the elevated temperature. The experiment was fully cross-designed with eight esfenvalerate concentrations (0, 0.001, 0.01, 0.0316, 0.1, 0.316, 1.0 and 3.16 μg/L) × two temperature levels (20 and 25 °C) × two food conditions (high and low food), resulting in 32 treatments. In each treatment, we used 15 replicates, and the experiment was repeated 3 times. In treatments with insecticide exposure, daphnids were exposed to esfenvalerate for 24 h following a 7-day acclimation period to the corresponding food and temperature conditions (Fig. 1).

These daphnids were individually tested in small glass vessels containing 80 mL of the test solution. The test medium (ADaM) was changed three times a week, and the mortality was monitored throughout the experiment, with dead individuals being promptly removed. Neonates were counted during the medium changes. Organisms in the high food treatments were fed with 0.5×10^9 cells per individual per day in the first week, 1.15×10^9 cells per individual per day in the second week, and 1.35×10^9 cells per individual per day in the third and fourth weeks. In the low food treatments, organisms were fed 100 times less food (Shahid et al., 2019).

2.2. Selection of chemical and non-chemical stressors

To study the interaction between pesticides and non-chemical environmental stressors, the pyrethroid insecticide esfenvalerate (Chemical Abstracts Service (CAS) 66230-04-4, with a purity of 99.8%, HPC Standards, Cunnersdorf, Germany) was selected as a chemical stressor, and elevated temperature and food limitation as non-chemical stressors. We selected esfenvalerate because it has frequently been detected in agricultural streams and is allowed for agricultural practices in the EU until May 2026. Whereas, other type-II pyrethroids are permitted until the end of the decade (Database, 2024). Following the neonicotinoid ban, sales of pyrethroids have increased, suggesting that they are at least partially replacing neonicotinoids.

We applied esfenvalerate at the concentrations commonly found in the field, ranging from trace levels to 0.76 μg/L, as reported in previous studies (Bacey et al., 2005; Cooper et al., 2003; Liess et al., 1999; Munze et al., 2017). We used acetonitrile as the solvent to prepare the stock solution and ensured that the solvent concentration remained within the limits recommended by the Organisation of Economic Cooperation and Development (OECD) guidelines (OECD, 2000). Further dilutions were prepared in ADaM.

2.3. Chemical analyses

To verify the exposure concentrations, 250 mL of samples were collected from the different dilutions within 1 h of preparing the solutions and analyzed for esfenvalerate by SGS Analytics GmbH, Germany, using GC–MS. The median measured concentration of each nominal concentration ranged within the acceptable boundaries (±20%). The lowest concentration (0.001 μg/L) was below the detection limit, and validated by the higher concentration used for the dilution.

2.4. Statistical analysis comparison of models

Data analyses and illustration production was done with the software R Studio (version 2024.4.1.748) (RStudio, 2024) and R (version 4.4.0) (R Core Team, 2024). To increase the number of replicates and the robustness of the analyses, we pooled all experimental runs and used this data set for subsequent analyses. To compare the lethal concentrations (LC_{50} and LC_{10}) of esfenvalerate across different food levels (high and low food) and temperature conditions (20 and 25 °C), we employed a log-logistic model for concentration-response relationships (Ritz and Streibig, 2005). We considered five-parameter log-logistic function LL.5 (Eq. 1), which facilitates asymmetric fit of the data, providing better modeling of the observed responses.

$$N(C) = c + \frac{d - c}{\left(1 + (C/e)^{b}\right)^{f}} \tag{1}$$

where the population size *N(C)* represents surviving organisms as a function of the toxicant concentration *C*. The parameter $b > 0$ controls the shape of the curve, while, *c* and *d* indicate the lower and upper limits of *N(C)*, respectively. The parameter $e > 0$ indicates the scale parameter, and *f* controls asymmetry. In this case, the lower limit *c* is fixed at 0, indicating no survival, and the upper limit *d* is fixed at the population response observed in the control group. To compare the LC_{50} values under different stress conditions, we employed repeated measures ANOVA, followed by pairwise *t*-tests.

To better understand the interaction between various stressors, we predicted the combined effects of (i) elevated temperature and food limitation, (ii) elevated temperature and esfenvalerate, (iii) esfenvalerate and food limitation, and (iv) all stressors together (see Fig. 1). For (i), we compared the mortality of treatments exposed to elevated temperature and food limitation separately with the mortality of a treatment where organisms were exposed to both stressors simultaneously. Whereas, for (ii) and (iii), we compared lethal concentrations of esfenvalerate at reference temperature versus elevated temperature and high food versus low food condition. To investigate the combined effects of esfenvalerate, sub-optimal temperature (25 °C) and food limitation (iv), we compared the LC values of all setups under high temperature (25 °C) and low food with a control setup at 20 °C, without food limitation (representing the best-case scenario, see Fig. 1).

For predicting the joint impact of stressors, we employed two conventional approaches (CA and EA), along with a recently developed approach SAM. For the effect addition approach, the joint effect was predicted by the equation (Eq. (2) (Bliss, 1939),).

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} \left(1 - E(c_i)\right) \tag{2}$$

where $E(c_{mix})$ is the joint effect of $E(c_i)$ stressors.

For the Concentration Addition (CA), the prediction was based on the following equation (Eq. (3), Loewe and Muischnek (1926))

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i}\right)^{-1} \tag{3}$$

where ECx_{mix} represents the sum of concentrations of all stressors, *pi* represents the relative fraction of stressor *i*, and *ECxi* is the concentration of the stressor *i* that induces × % effect. Since CA is typically used to predict the effects of toxicant mixtures, we adapted it by converting the effects of non-chemical stressors like elevated temperature and food limitation into equivalent normalized concentrations of esfenvalerate.

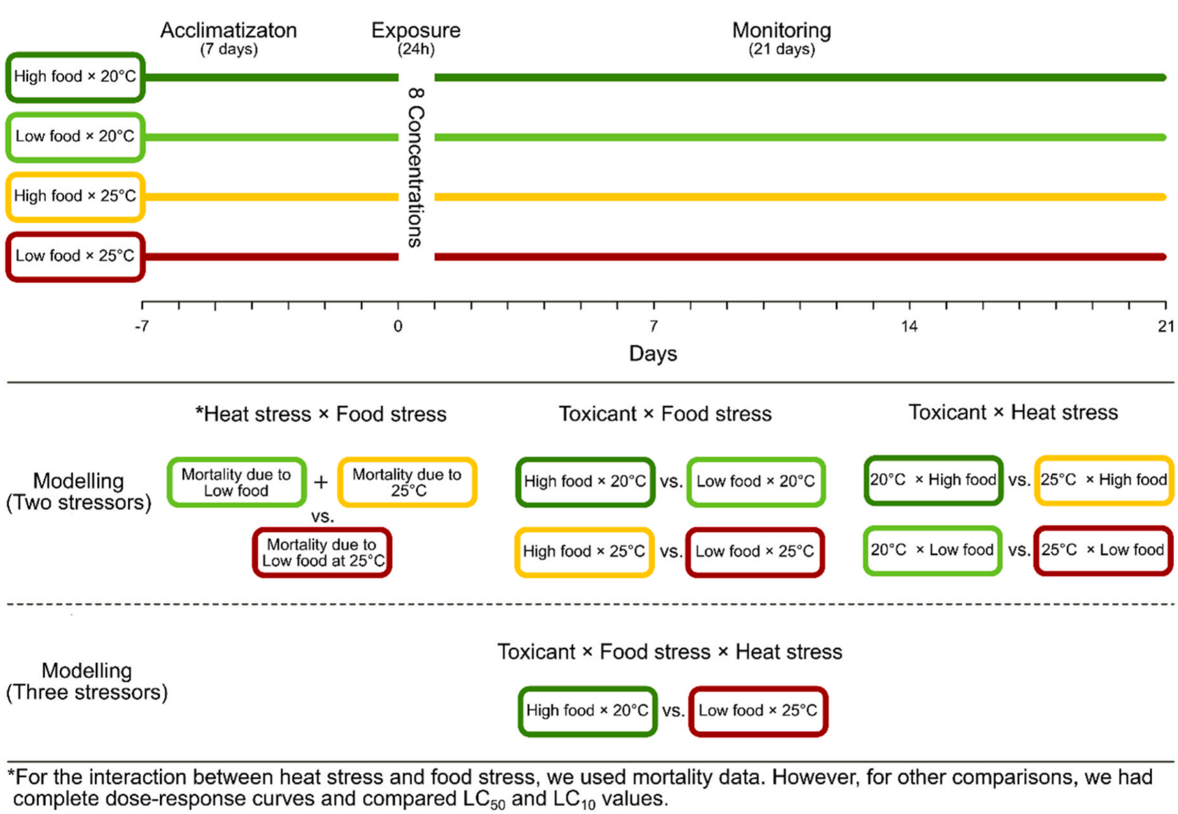

Fig. 1. Overview of the experimental design. *Daphnia magna* were acclimatized to different food levels (high and low) and temperature conditions (20 °C and 25 °C) for 7 days. They were then exposed to a range of esfenvalerate concentrations (0–3.16 μg/L) for 24 h under four different conditions: two food levels (high and low) and two temperatures (20 °C and 25 °C). Subsequently, the interactions between two and three stressors were predicted using CA, EA and SAM.

For the Stress Addition Model, stress related survival was calculated as the reduction of general stress capacity for the population by all stressors. For this, we applied the equation (Eq. (4)) describing the distribution of general stress capacity within the population (Liess et al., 2016).

$$N(S) = 1 - \int_0^s p(S)\, dS \tag{4}$$

where N(S) = 1 (100% survival) for the general stress S = 0 and N(S) = 0 (0% survival) for the general stress S ≥ 1. The total general stress "S" was calculated as the sum of general stress levels S_i of all independently acting stressors (for details, see Liess et al. (2016)).

$$S = \sum S_i \tag{5}$$

For the combined effects of stressors in terms of lethal concentrations (LC_{50} and LC_{10}), we employed EA, CA, and SAM models using a web-based application (Indicate, version 2.2.1; http://www.systemecology.eu/indicate/).

To evaluate the predictive accuracy of these models, we divided predicted LC_x values by the observed LC_x values and calculated the model deviation ratios (MDR, Eq. 6). An MDR value close to 1 (MDR ≈ 1) indicates better accuracy of the model in quantifying the combined effects of multiple stressors.

$$\text{MDR} = \frac{\text{Predicted Lethal Concentration}}{\text{Observed Lethal Concentration}} \tag{6}$$

We used effect addition as a null model for the combined effects of stressors. An MDR value of LC_{50} concentration <0.5 indicated an antagonistic response, whereas, an MDR >2 suggested synergism (Cedergreen, 2014). Due to potentially higher variability within the range of LC_{10}, we shifted the threshold for identifying synergism to an MDR value of 5 for LC_{10}.

3. Results

3.1. Additive effects of elevated temperature and food limitation

In the absence of esfenvalerate, elevated temperature and food limitation interacted according to the principle of effect addition (EA). After 21 days, elevated temperature alone caused 12% mortality, while starvation alone caused 20% mortality. However, when both stressors were combined, the mortality rate increased to approximately 29%, indicating the additive effects of both stressors (MDR = 0.9).

3.2. Interaction between elevated temperature and esfenvalerate

To quantify the interaction between esfenvalerate and elevated temperature, we compared the toxicity of esfenvalerate at reference and elevated temperature (20 and 25 °C) under high and low food conditions relative to their corresponding controls (high and low food controls, see Fig. 1). Under high food conditions, elevated temperature did not cause any synergistic interaction with esfenvalerate (Table 1). However, under low food conditions, strong synergism – well above an MDR of 2 – was observed on day 14 (MDR = 3.66) and day 21 (MDR = 4.53) (Table 1, Fig. 2).

3.3. Interaction between esfenvalerate and food limitation

To reveal the interaction between esfenvalerate and food limitation, we compared the toxic effects of esfenvalerate under high and low food conditions at two different temperatures (20 and 25 °C) relative to their corresponding controls (i.e., controls at 20 and 25 °C). Using effect addition (EA) as a null model, we observed synergistic interactions between esfenvalerate and food stress (Fig. 3, Table 1). At a reference temperature of 20 °C, food limitation increased the sensitivity of *Daphnia magna* to esfenvalerate and caused synergism shown by the model deviation ratio (MDR) of 3.35 on day 7. The strength of synergism was slightly increased over time (day 14: 4.46). Nevertheless, there was not much difference between day 14 and day 21. Overall, food limitation was the strongest stressor, significantly reducing the tolerance (LC_{50}) of daphnids to esfenvalerate (Table 2, repeated measures ANOVA, *p*-value <0.05).

A similar trend was observed at the elevated temperature (25 °C). However, the long-term interaction between esfenvalerate and food limitation showed stronger effects at the elevated temperature. Initially at day 7, the interaction between esfenvalerate and food stress was slightly less synergistic (MDR = 2.2) compared to synergism at 20 °C (MDR = 3.35) at day 7. However, the strength of synergism increased over time and showed up to ~ 6-fold higher MDR values on day 21 (MDR = 12). Thus, the synergistic interaction between food limitation and esfenvalerate at elevated temperature (25 °C) was 3.6-fold stronger as compared to synergism at reference temperature (20 °C).

3.4. Interaction between multiple stressors

For cumulative effects of esfenvalerate and both non-chemical environmental stressors, we compared all treatments of low food availability and elevated temperatures to control with high food at the reference temperature (best case scenario). Our results indicate that, compared to high food and reference temperature, the combination of food limitation and elevated temperature substantially increased the sensitivity of *D. magna* to esfenvalerate (Fig. 4, Table 2). The MDR values calculated for the LC_{50} were 2.4, 16.3, and 18.9 on days 7, 14 and 21, respectively (Fig. 4, Tables 1 and 2), indicating 8-fold latent synergism. On the same days, the MDR values calculated for the LC_{10} were even higher: 8, 77, and 130, respectively (Table 1, Table 2), showing double latent synergism compared to LC_{50} (16-fold).

3.5. Accuracy of predictive models

A comparison between EA, CA, and SAM for predicting multiple stress effects using the MDR (the ratio between predicted and observed LC_{50} and LC_{10} values) shows that SAM considerably outperformed the other models, showing the best fit to the modelled curves (Figs. 2–4) and the lowest MDR values (Table 1). Overall, prediction by SAM was substantially better than EA and CA. For instance, for the prediction of LC_{50}, EA and CA underestimated the combined effects of esfenvalerate and food limitation by up to a factor of 12 and 18, respectively. When exposed to multiple stressors including esfenvalerate, food limitation and elevated temperature, the underestimation of the combined effect by EA and CA reached up to 19 and 18.32 times, respectively. On average, the SAM model outperformed both conventional models by 5 times in predicting the latent synergism of esfenvalerate and food limitation, and by 7 times for the latent combined effects of all three stressors.

For predicting the LC_{10}, which is more relevant for assessing long-term effects in the field, we considered an MDR >5 to indicate synergism. Again both conventional models EA and CA underestimated the combined effects of esfenvalerate and food limitation by up to a factor of 33 and 50, respectively. In contrast, SAM performed considerably better (Table 1), with a slight overestimation of the combined effects. Except in one case, the MDR values of SAM did not deviate by more than 5 (the threshold for synergism), and the predictions were on average 5 times more accurate.

For the combined effects of multiple stressors including esfenvalerate, food limitation and elevated temperature, the underestimation by EA and CA reached up to 130 and 84 times, respectively (Table 1). SAM showed significantly better predictive accuracy, with predictions only overestimating combined effects of up to a factor of 3. On average, the SAM approach performed 40 times better than both conventional

Table 1
Interaction of esfenvalerate with environmental stressors and prediction of combined effects on LC_{50} and LC_{10}. The combined effects of stressors are predicted using different models, and interactions are considered synergistic when the Model Deviation Ratio (MDR) exceeds 2 for LC_{50} and 5 for LC_{10}.

1st Stressor	2nd Stressor	3rd Stressor	Reference Condition	Day	MDR		
					EA	CA	SAM
Combined effects of stressors on LC_{50}							
Toxicant	Food limitation	–	High food	7	**3.35**	2.94	0.96
			20 °C	14	**4.46**	4.04	0.84
				21	**4.15**	3.92	0.79
			High food	7	**2.19**	2.24	0.49
			25 °C	14	**11.13**	12.46	1.16
				21	**12.01**	18.31	0.40
Toxicant	Elevated	–	High food	7	1.09	1.00	0.47
	Temperature		25 °C	14	1.46	1.34	0.46
				21	1.57	1.48	0.49
			Low food	7	0.71	0.71	0.71
			25 °C	14	**3.66**	3.75	0.74
				21	**4.53**	4.76	0.74
Toxicant	Food limitation	Elevated	High Food	7	**2.39**	2.10	0.69
		Temperature	20 °C	14	**16.31**	14.97	2.41
				21	**18.83**	18.32	2.48
Combined effects of stressors on LC_{10}							
Toxicant	Food limitation	–	High food	7	**9.62**	5.69	0.88
			20 °C	14	**6.86**	4.02	0.29
				21	**5.95**	3.95	0.25
			High food	7	4.31	3.90	0.25
			25 °C	14	**32.99**	31.32	0.47
				21	**22.27**	49.71	0.04
Toxicant	Elevated	–	High food	7	1.84	1.31	0.37
	Temperature		25 °C	14	2.33	1.57	0.26
				21	**5.85**	4.33	0.62
			Low food	7	0.82	0.82	0.82
			25 °C	14	**11.19**	10.06	0.52
				21	**21.90**	20.09	0.69
Toxicant	Food limitation	Elevated	High Food	7	**7.91**	4.68	0.72
		Temperature	20 °C	14	**76.80**	43.68	2.23
				21	**130.3**	84.04	3.03

Note: For the interaction of food stress and elevated temperature, we used mortality instead of LC_{50}. Using effect addition (EA) as a null model, we observed additive effects of food limitation and elevated temperature (25 °C), with MDR values of 0.71 at day 7, and 0.92 at 14 and 21.

Table 2
The lethal concentrations (LC_{50} and LC_{10}) of esfenvalerate across different food levels (high and low food) and temperature conditions (20 and 25 °C).

Temperature °C	Food level	Day	Observed LC_{50} μg/L	Observed LC_{10} μg/L
20 °C		7	0.687	0.175
	High food	14	0.519	0.121
		21	0.452	0.096
		7	0.205	0.018
	Low food	14	0.116	0.018
		21	0.109	0.016
25 °C		7	0.630	0.095
	High food	14	0.354	0.052
		21	0.288	0.016
		7	0.287	0.022
	Low food	14	0.032	0.002
		21	0.024	0.0007

models in predicting the latent (day 14 and 21) combined effects of all three stressors.

To identify the association between general stress and synergism, we used the SAM framework and calculated total general stress by adding individual stress posed by esfenvalerate, food limitation and elevated temperature (see methods). We revealed that the synergistic interactions between stressors were increasing with higher total stress (Null model EA: Fig. 5, $R^2 = 0.76$).

4. Discussion

Indeed, it's well established that environmental stressors can substantially alter the responses of organisms to toxicants (Laskowski et al., 2010; Macaulay et al., 2021a; Shahid et al., 2019; Siddique et al., 2021; Verheyen et al., 2022), yet the effects of their interactions in complex combinations are not well understood. In the present study, we investigated how food limitation and warming – relevant environmental stressors under climate change – interact with a pyrethroid insecticide esfenvalerate. We observed that low food availability exacerbated the long-term effects of esfenvalerate. Furthermore, the synergistic interaction between esfenvalerate and food limitation was intensified by warming. However, in the absence of the toxicant, non-chemical stressors acted according to the principle of effect addition (EA).

Food limitation was a strong environmental stressor that synergistically increased the effects of esfenvalerate by a factor of 3.3, which was further increased over time, indicating delayed synergism (Table 1). Such latent increases in synergistic effects between chemical and non-chemical stressors (System stress, emergence stress) were quantitatively described by Liess and Gröning (2024). The strong effect of esfenvalerate under food limitation can be attributed to metabolic depression resulting in limited energy budget for physiological defences against stress (Sibly, 1999). Also, Dinh et al. (2016) reported a lower energy budget and reduction in immune function in starved damselfly larvae. During periods of starvation, organisms often reduce their metabolic activity as an adaptive mechanism (Storey, 2015). This strategy supports to minimize the depletion of energy reserves and survive longer. Several studies have reported that food limitation increases the toxicity of pesticides and metals in invertebrates (Barry et al., 1995; Beketov and Liess, 2005; Pieters et al., 2005; Shahid et al., 2019; Shahid et al., 2021).

In our study, elevated temperature alone did not result in a synergistic interaction, as indicated by MDR values of 0.7–0.9. Although exposure to extreme temperatures can lead to physiological

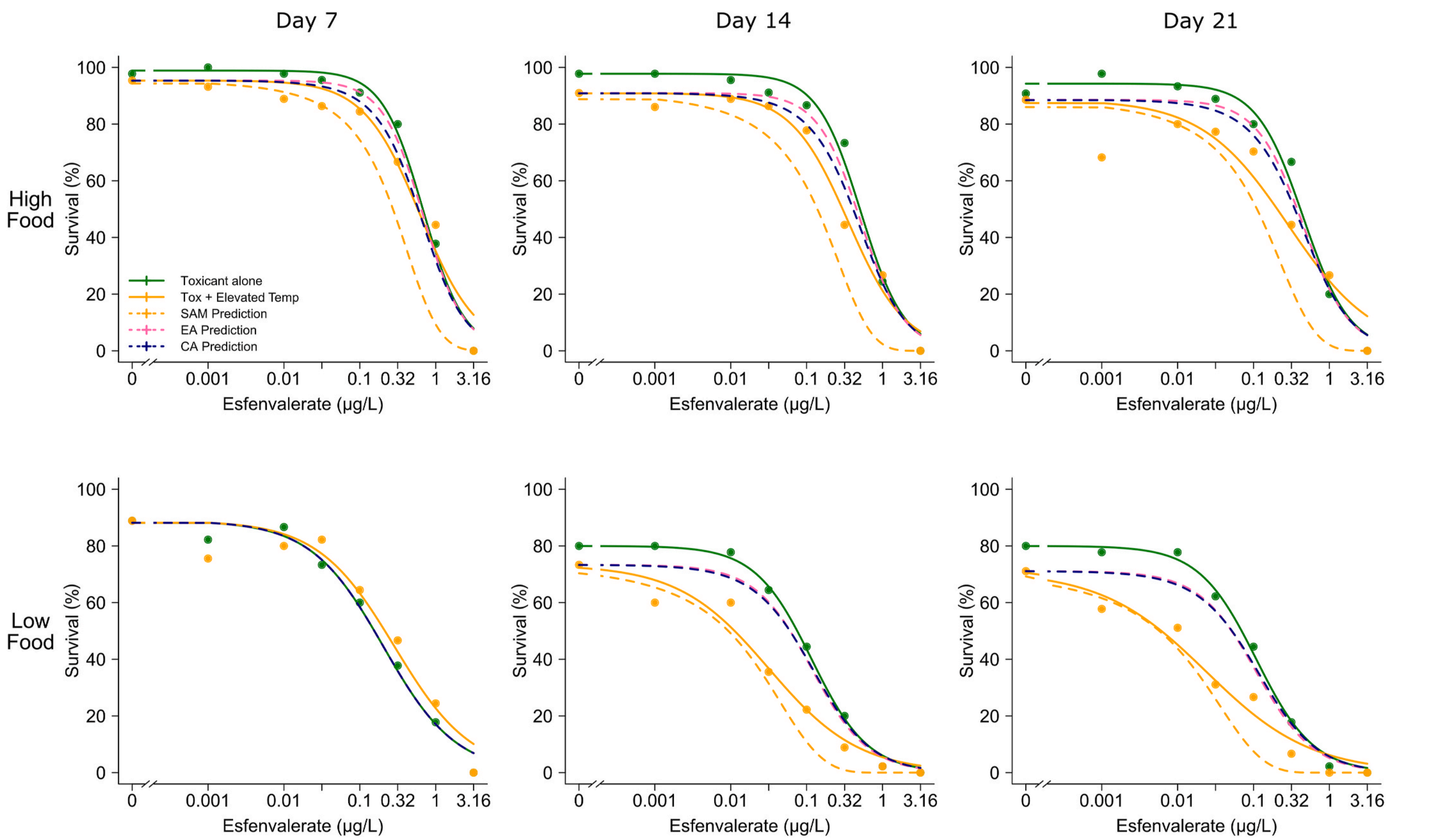

Fig. 2. Survival of *Daphnia magna* exposed to insecticide esfenvalerate at different temperature regimes (20°C and 25°C). Dose-response relationships are displayed for day 7, day 14 and day 21 under high and low food conditions. The solid green line represents survival without additional stress, whereas the orange solid represents survival at elevated temperature. The dashed lines represent modelled dose-response relationships under multiple stress conditions. The prediction by SAM is represented by the orange dashed line, whereas, EA by pink, and CA by blue dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

dysfunctions and potentially result in mortality (Garrabou et al., 2009; Mislan and Wethey, 2015; Petter et al., 2014), the nature of their interactions with other stressors, and cumulative impacts from multiple stressors are not consistent. For example, Janssens et al. (2014) observed stronger impacts of the organophosphate insecticide chlorpyrifos on the damselfly populations (*Ischnura elegans*) at 30 °C compared to the reference temperature of 20 °C. In contrast, Op de Beeck et al. (2018) reported stronger effects of the same toxicant on the same species at 20 °C compared to 24 °C. The authors of the latter investigation suggested that the stronger effects observed could be due to the slower degradation of the toxicant at lower temperature. Nevertheless, Janssens et al. (2014) showed higher effects at 30 °C despite the expected faster degradation of the toxicant. This discrepancy could be explained by the overriding effect of heat stress, which may negate the anticipated benefits of faster degradation at higher temperatures. Conversely, a meta-analysis on multiple stressors concluded that dominant stressors such as contamination, override biological effects of elevated temperatures in freshwater ecosystems (Morris et al., 2022).

It is suggested that the negative impacts of elevated temperature become more severe in starved organisms (Adamo et al., 2012; Lee and Roh, 2010). However, contrary to this, we observed additive effects of elevated temperature and food limitation. This might be attributed to the fact that mild heat stress tends to upregulate metabolic rates in organisms, whereas stronger heat stress can induce metabolic depression (Frederick et al., 2022; Sarup et al., 2016). Under such high-stress conditions, organisms dedicate all the available energy and metabolic capacity to survival, awaiting favourable conditions (Sokolova et al., 2012).

Elevated temperature appeared to exacerbate the synergistic interaction between food limitation and esfenvalerate exposure in our study. Under multiple stress conditions, the LC_{50} of esfenvalerate was reduced by a factor of 19, whereas, the LC_{10} was reduced drastically by a factor of 130 (Table 2). These surprising effects especially at LC_{10} underscore the importance of considering multiple stressors in ecological risk assessments of pesticides. Synergistic effects of these three stressors with different modes of action can be explained by their interaction at various physiological levels in *Daphnia magna*. For instance, elevated temperature increases metabolic rates (Frederick et al., 2022), leading to greater energy demands, which could compromise the organism's ability to detoxify chemicals (Sokolova, 2021) or cope with limited food availability. Additionally, temperature stress might weaken the immune system, making daphnids more vulnerable to toxicant exposure. Thus, cumulative effects likely disrupt homeostasis, leading to synergistic rather than additive response.

Only a few investigations have studied the complexities of such multiple-stressor combinations. For instance, Janssens and Stoks (2013) investigated the combined effects of chlorpyrifos, temperature, and food level on the damselfly *Enallagma cyathigerum*. The concentration of chlorpyrifos was high enough to cause a drastic reduction in survival (<50) at 24 °C. On the other hand, temperature and food stress were not enough strong to cause mortality. Overall, the combined effects of these stressors were mostly interactive and sometimes reversed compared to the effects of individual environmental stressors. Accordingly, it is not possible to predict the combined effects of multiple-stress based on individual environmental stressors. In our study, however, the combined effect of all three stressors was synergistic and could be predicted based on the individual stress calculated by the SAM framework.

Establishing a "common currency" to integrate different stressors

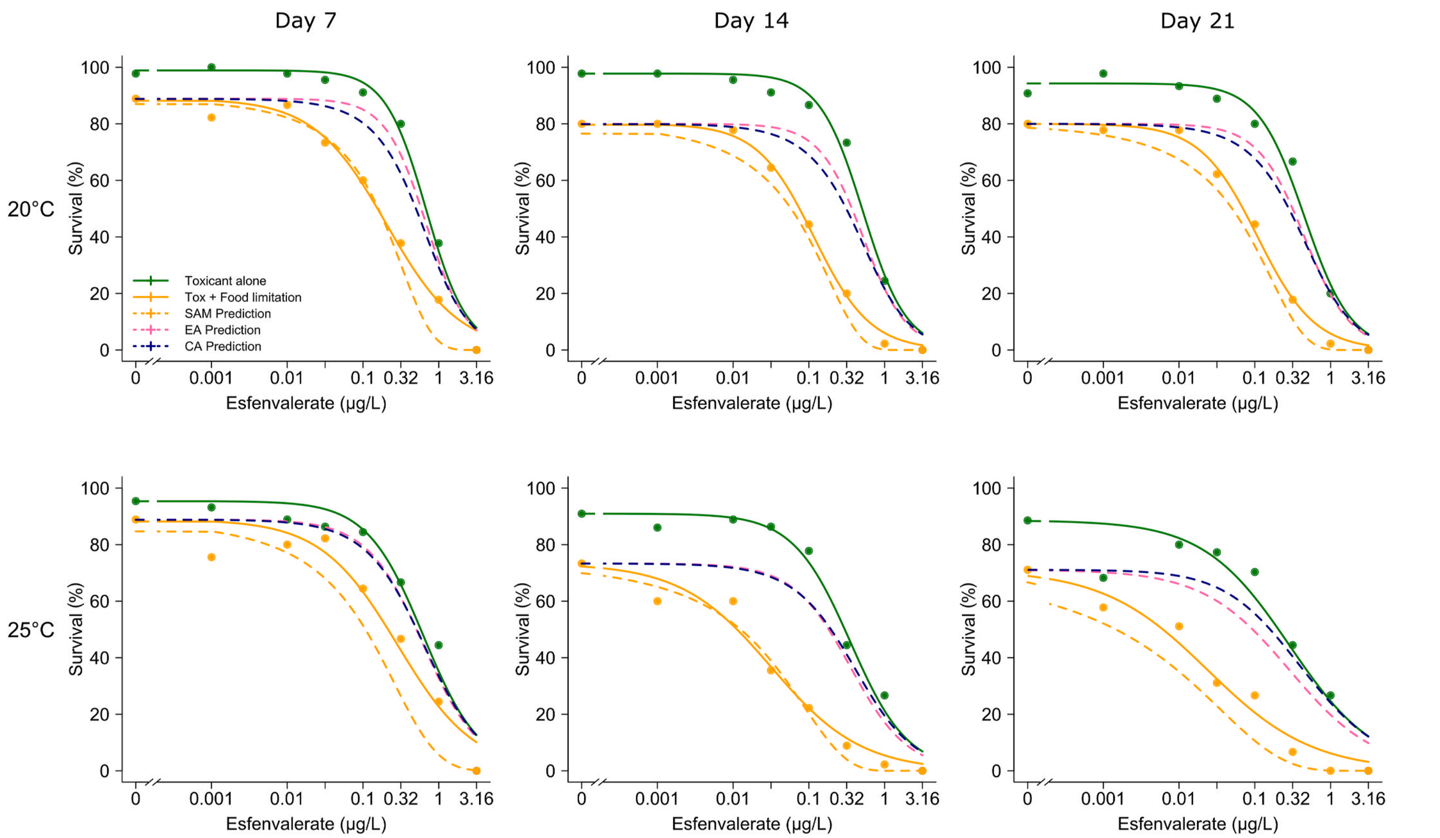

Fig. 3. Survival of *Daphnia magna* exposed to insecticide esfenvalerate under limited food availability. Dose-response relationships are displayed for day 7, day 14 and day 21 at different temperature regimes (20 °C and 25 °C). The solid green line represents survival without additional stress, whereas the orange solid represents survival under low food availability. The dashed lines represent modelled dose-response relationships under multiple stress conditions. The prediction by SAM is represented by the orange dashed line, whereas, EA by pink, and CA by blue dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

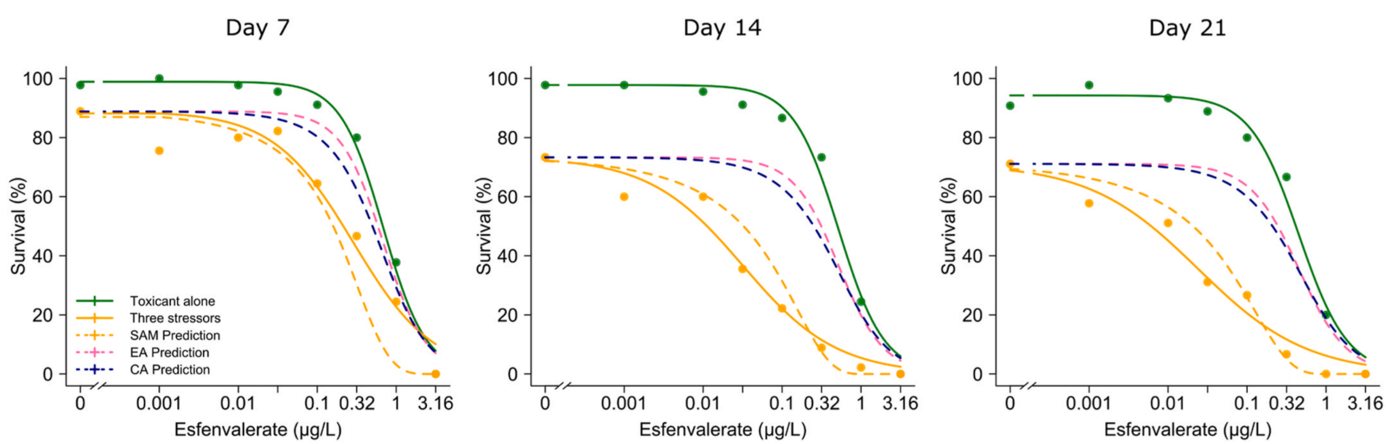

Fig. 4. Survival of *Daphnia magna* exposed to insecticide esfenvalerate under limited food availability and temperature stress (25°C). Dose-response relationships are displayed for day 7, day 14 and day 21. The solid green line represents survival without additional stress, whereas the orange solid represents survival under low food availability. The dashed lines represent modelled dose-response relationships under multiple stress conditions. The prediction by SAM is represented by the orange dashed line, whereas, EA by pink, and CA by blue dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was a critical challenge in predicting the interactive effects of multiple stressors (Liess et al., 2016; Segner et al., 2014). According to the Stress Addition framework, each organism has a "general stress capacity" against stressors (Liess et al., 2016), and accordingly, different stressors can be transformed into general stress levels. Using the SAM framework, we calculated individual stress induced by additional stressors (food limitation and elevated temperature) and aggregated them to determine the total general stress. Each stressor diminished the general stress capacity of individuals, thereby increasing synergism with increasing total general stress (Fig. 5).

Overall, conventional models (CA and EA) underestimated the interaction between (i) elevated temperature and esfenvalerate, (ii) food limitation and esfenvalerate, (iii) and multiple stressors including elevated temperature, food limitation and esfenvalerate (multiple-stress

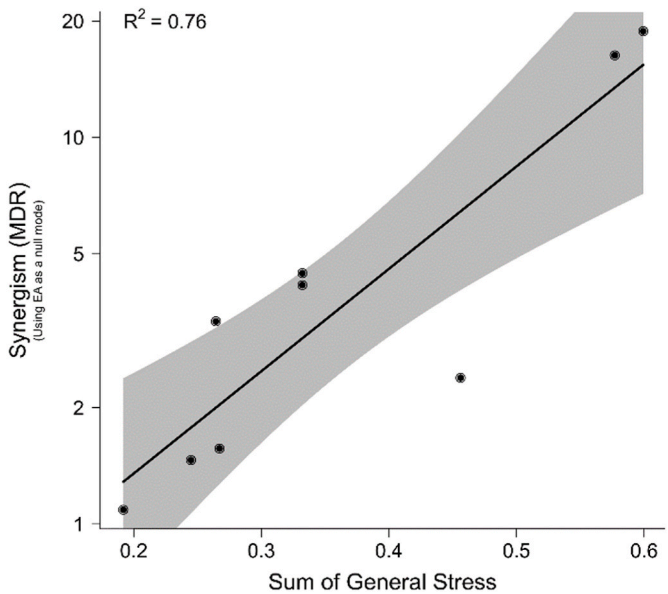

Fig. 5. Association between the sum of general stress and the strength of synergism at different time points: Combined stress of multiple stressors including food stress and elevated temperature significantly increased their sensitivity to esfenvalerate (linear regression, adjusted $R^2 = 0.76$, $F = 45.4$, residual d.f. $= 7$, $p < 0.001$).

conditions; Table 1, Figs. 2–4). Since conventional models (CA and EA) quantify cumulative effects by adding concentrations or effects, they inherently use an additive approach (Bliss, 1939; Loewe and Muischnek, 1926), and therefore, do not adequately capture the complex interactions among multiple stressors. Whereas SAM predicted the lethal concentrations (LC_{50} and LC_{10}) substantially better than CA and EA in nearly all cases (Table 1, Figs. 2–4). Up until now, SAM has successfully been employed to quantify the synergistic interaction between chemical and non-chemical environmental stressors (Liess et al., 2016; Liess and Gröning, 2024; Shahid et al., 2019; Siddique et al., 2021), toxicant mixtures (Shahid et al., 2019), and multiple stressors including pesticide adaptation in *Gammarus pulex* (Shahid et al., 2024).

5. Conclusions

Extrapolation from our results suggests that increasing temperature from global warming can drastically increase the synergistic interaction between chemical and non-chemical environmental stressors, such as starvation. This implies that species already facing food limitations are likely to experience population declines, particularly in agricultural streams. Although predicting the combined impacts of multiple stressors has traditionally been challenging, we could successfully predict the combined effects of distinct stressors using the stress addition model (SAM).

CRediT authorship contribution statement

Naeem Shahid: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft preparation, Writing – review & editing. **Ayesha Siddique:** Conceptualization, Investigation, Writing – review & editing. **Matthias Liess:** Conceptualization, Guided the analytical cognition process, Writing – review & editing, Funding acquisition.

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.

Acknowledgements

We thank Safa Khouaja from the Brandenburg University of Technology Cottbus-Senftenberg (BTU) for supporting lab experiments. We also thank Oliver Kaske from the Department of Ecotoxicology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany, for his support in maintaining the culture of *Daphnia magna*. This investigation is supported by the Helmholtz Research (POF IV, Topic 9 “Healthy Planet”) and the European Union's Horizon Europe research and innovation programme under grant agreement No. 101057014. This publication reflects only the author's view, and the European Commission is not responsible for any use that may be made of the information it contains.

Data availability

Data will be made available on request.

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