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Latent pesticide effects and their mechanisms

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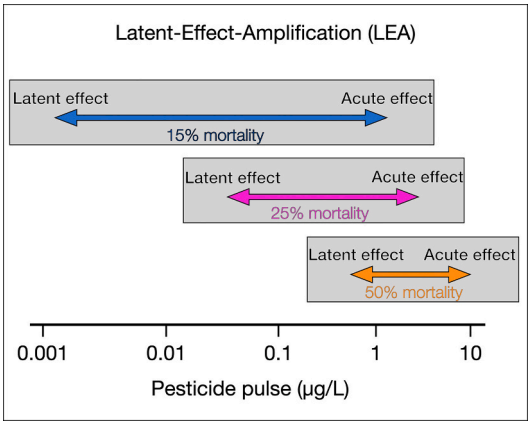
HIGHLIGHTS

- One hour pesticide contamination induced latent effects in an aquatic insect.
- Latent, possibly endocrine, effects dominated especially at low concentrations.
- Immediately after contamination the pesticide-response relationship is monotonic.
- Then latent stress and compensation developed at population and community level.
- As a result, a tri-phasic hormetic response pattern developed.

GRAPHICAL ABSTRACT



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ABSTRACT

Short pulses of toxicants can cause latent effects that occur long after the contamination event and are currently unpredictable. Here, we introduce an analytical framework for mechanistically predicting latent effects considering interactive effects of multiple stressors and hormetic effect compensation. We conducted an extensive investigation using high temporal resolution microcosm data of the mayfly *Cloeon dipterum* exposed to the pyrethroid pesticide esfenvalerate for 1 h. For 6 pesticide concentrations and 3 food levels we identified daily general stress information and predicted their synergistic interactions using the Stress Addition Model (SAM). Our analysis revealed that, especially at low concentrations, latent effects contributed most to the overall effect. At low concentrations ranging from 1/100 to 1/10,000 of the acute LC_{50}, resulting in a 30–15 % mortality, latent effects prevailed, accounting for 92 % to 100 % of the observed effects. Notably, the concentration causing 15 % mortality 29 days post-exposure was 1000 times lower than the concentration causing the same mortality 4 days post-exposure, emphasizing the time-dependent nature of this Latent-Effect-Amplification (LEA). We identified both acute mortality and latent effects of pesticides on emergence. Furthermore, we observed pesticide-induced compensation mechanisms at both individual and population levels, transforming the initial monotonic concentration-response relationship into a hormetic, tri-phasic response pattern. Combining these processes

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enabled a quantification of the underlying causes of latent effects. Our findings highlight that short-term pesticide exposures can lead to latent effects of particular significance, especially at low effect concentrations.

1. Introduction

Assessing the long-term effects of toxicants is a critical aspect of environmental effect assessment. With regard to aquatic standard test systems, life cycle test systems with *Daphnia magna* and *Chironomus riparius*, for example, have been established for the assessment of chronic contamination (EFSA, 2013). However, also short pulse contamination scenarios are especially relevant and current. For example, precipitation-initiated pulse contamination is characteristic of emissions from agriculture and urban areas. For such scenarios, there is no established test system that combine pulsed contamination with extended observation periods (EFSA, 2013). Consequently, existing methodologies rely on extrapolating results from acute test systems to predict long-term effects. In the first tier effect assessment, for example, it is commonly assumed that no chronic effects occur in the field at 1/100 of the acute LC_{50} (EFSA, 2013). Also in the derivation of Predicted No Effect Concentrations (PNECs) through Species Sensitivity Distributions (SSDs), acute LC_{50} values are generally used without long-term observation periods (Kooijman, 1987).

Empirical studies have shown, however, that current effect assessment fails to protect vulnerable populations at the ecosystem level. Examples include pesticide effects in agricultural streams from Australia (Beketov et al., 2013), Europe (Liess et al., 2021; Liess and Von Der Ohe, 2005; Schäfer et al., 2012), North America (Chiu et al., 2016) and South America (Hunt et al., 2017). The challenge in extrapolating from the laboratory to the field situation lies in the complex extrapolation from acute LC_{50} values to chronic PNEC values, which include both temporal and effect size dimensions. Numerous studies investigating the long-term effects of short-term pollutant exposure have revealed persistent latent effects, extending well beyond the initial contamination event, overarching the lifespan of affected individuals. For instance, lethality in various insect species was observed weeks or months after brief exposure to the pyrethroid insecticide esfenvalerate within single species test-systems (Beketov and Liess, 2005; Liess, 2002; Liess and Schulz, 1996). Also insecticide contamination within multi-species outdoor mesocosms revealed latent effects (Bhattacharyya et al., 2023; Bray et al., 2021; Liess and Beketov, 2011). Latent mortality was also documented in cases involving combined effects of environmental stressors in combination with toxicants, including metal exposure combined with warming (Debecker et al., 2017) and pesticide exposure combined with warming (Janssens et al., 2014). Studies combining esfenvalerate with UV radiation, food restriction and unfavourable temperatures also revealed latent effects that occurred at progressively lower concentrations as the environmental stressors increased in strength (Liess et al., 2019). Furthermore, latent sublethal effects have been identified, such as the disruption of pairing behaviour and reproductive output in the freshwater amphipod *Gammarus pulex*, following just a 1-hour esfenvalerate exposure, persisting for at least two weeks (Cold and Forbes, 2004).

Moreover, when examining low exposure concentrations, it becomes evident that concentration-response relationships are not simply monotonic but exhibit bi-phasic, non-monotonic, relationships, a phenomenon known as hormesis (Schulz, 1877). A comprehensive literature review has demonstrated that hormesis occurs across various chemicals, taxonomic groups, and endpoints (Calabrese et al., 1999; Stebbing, 1982). This concept of a non-monotonic concentration-response relationship has expanded to a tri-phasic relationship as depicted by Stebbing (Stebbing, 1982) and described in detail by Agathokleous (Agathokleous, 2022). At ultra-low pesticide concentrations ($\leq$1/100 of the acute LC50), a reduction in survival compared to controls has been observed, and the resulting tri-phasic relationship was mechanistically explained (Liess et al., 2019). Intriguingly, such deviations from monotonic dose-response relationships are not consistently considered in the risk assessments of governmental agencies like the European EFSA, US-EPA, or China's MEE (Agathokleous et al., 2022a).

The aim of the study was to develop a simple framework that mechanistically accounts for both acute and also latent/delayed effects in the time period following pulsed contamination. In addition, non-monotonic dose-response relationships were to be considered in the prediction.

2. Material and methods

2.1. Experimental setup

We re-analysed the data from Beketov and Liess (Beketov and Liess, 2005). Here, we provide a concise overview of the experimental conditions, with more comprehensive details available in the original paper.

After a brief 1-h exposure to esfenvalerate, ten third and fourth instar larvae of *Cloeon dipterum* (Baetidae, Ephemeroptera) were rinsed and transferred to triplicated indoor two-liter microcosms containing insecticide-free water. These microcosms maintained the individuals until their emergence, with varying levels of intraspecific competition resulting from high-, medium-, and low-food treatments. Food was provided in the form of a mixture of dried leaves from pendent white birch (*Betula verrucosa*) and willow (*Salix* sp.), with different food quantities: high food (20 mg/microcosm/day, ad libitum), medium food (20 mg/microcosm every second day, slightly below ad libitum), and low food (20 mg/microcosm every fifth day, representing strong food deficiency). To maintain favourable water chemistry, 50 % of the microcosm water was exchanged with fresh pond water every week. Larval survival following the one-hour contamination, emergence success, body weights of adult mayflies, female fecundity, and total egg production, were recorded at day 4, 8, 15, 22, and 29, when all individuals had either emerged or died. Esfenvalerate (A-alpha isomer) was applied as an emulsifiable concentrate (Sumi-alfat; Sumitomo, Osaka, Japan) containing 50 g/L of active substance. The 1-hour exposure included the following concentrations: control, 0.001, 0.01, 0.1, 1, 10, and 100 μg/L. Actual exposure concentrations were determined after solid-phase extraction of 1-liter volumes using Bakerbond PolarPlus C18 columns (Baker, Philipsburg, NJ, USA) and measured with a gas chromatograph (model 6890; Hewlett-Packard, Avondale, PA, USA) coupled with a mass spectrometer detector (model 5972; Hewlett-Packard). The measured concentrations closely matched the nominal concentrations ($\leq$10 %).

Information that are relevant to consider in future investigations to extend the long-term perspective of pulsed contamination events include the evaluating of transgenerational effects as well as the consequences of pesticide adaptation present in field populations of non-target organisms (Shahid et al., 2018). Trade-off processes related to pesticide adaptation may increase long-term effects under multiple stress conditions (Siddique et al., 2021).

2.2. Calculations using the Stress Addition Model (SAM)

The Stress Addition Model (SAM) approach (Liess et al., 2016) was employed to identify general stress components responsible for individual mortality. The magnitudes of these stress components were combined according to SAM principles to derive the total general stress, with the following assumptions - also detailed in Liess et al. (2016, 2019):

(i) Each individual possesses a general stress capacity, symmetrically distributed over a finite interval [0, 1], to tolerate various stress types. We assume that stress-dependent population sensitivity follows the same distribution. Individuals with a stress capacity below a given stress level S will die, whereas individuals with a stress capacity above a given stress level will survive. Hence, stress-dependent population sensitivity is parameterized by the following beta distribution:

$$p(S) = \frac{1}{B(p,q)} S^{p-1}(1-S)^{q-1} \quad (1)$$

where p(S) represents the density probability of individuals to tolerate a general stress S, p and q are the non-negative shape parameters of the distribution and B (p, q) is the beta function, which is a normalization constant to ensure that the total probability integrates to 1. We postulated symmetry of the individual stress capacity (p = q). The parameters were set to p = q = 3.2, which resulted in the best fit between the observed and predicted LC 10 and LC 50 shifts in the 23 experimental study pairs in Liess et al. (2016). The integral of the density function gives the population size N under non-stress conditions:

$$N = \int_0^1 p(S)ds = 1 \quad (2)$$

The stress-dependent survival is calculated as

$$N(S) = 1 - \int_0^1 p(S)ds \quad (3)$$

where N(S) = 1 (100 % survival) for the general stress S = 0 and N (S) = 0 (0 % survival) for the general stress S ≥ 1.

(ii) The SAM presumes that each unit of stress from a given stressor can be converted to a general stress level using stress-related mortality as a linking factor. For instance, if a temperature stress or toxicant stress causes a mortality of 10 %, then the general stress level is given by the 10 % quantile of the beta distribution in Eq. (1).

(iii) The SAM assumes that the general stress levels of independent stressors are additive, with the sum determining the total general stress exerted on a population. The total general stress S is given as the sum of the general stress levels S_i of all stress components identified in Fig. 3 over time (Toxicant stress, System-Stress (SyS), Interaction stress, Emergence stress) (Eq. (4)). This stress addition results in a synergistic interaction of stressors when compared to the null model of effect addition (Bliss, 1939). We also added a temporal component, assuming that stress is cumulative over time. For our model, the total stress (converted from survival) was decomposed into toxicant stress, SyS, interaction stress and emergence stress. Larval related toxicant, system, and interaction stress were assumed to act until day 22 when larvae were present in the water. After day 22 only emergence/toxicant related stress was effective.

$$S = \sum S_i \quad (4)$$

The resulting survival of the population exposed to the general stress S can be determined by applying Eq. (3). Conversely, its inverse can be used to determine general stress from observed survival. A more detailed description of the approach can be found in the original publication (Liess et al., 2016). We provide an R-script in the supplementary information containing the data and the approach used. In addition, a calculator within the INDICATE program package (https://www.systemecology.de/indicate/) can be applied to facilitate own calculations.

2.3. Identification of individual stress components

The individual stress components were sequentially fitted to the experimental data using the following approach:

(i) Toxicant stress: A general toxicant concentration-response curve was first calculated by averaging the results of the ECx-SyS model (Liess et al., 2019) for 4, 8, 15, and 22-day survival from the high-food experiment data. The resulting dose-response curve was then scaled for each time step to minimize residuals in the modelled survival. Toxicant stress was fitted based on the high-food setup and subsequently applied to all other setups with lower food concentrations.

(ii) System-Stress (SyS): SyS was fitted to the experimental data to further reduce residuals in the data from the high-food experiment. As detailed in Liess et al. (Liess et al., 2019), SyS was identified as a relevant stressor on the individual level, in the absence of intraspecific competition and toxicant stress. This stress is highest in the control and decreases with increasing concentrations. Temporal changes in SyS were also considered. Additionally, we revealed the temporal change of SyS as presented in Fig. 3. The SyS was fitted based on the high-food setup and applied to all other setups with lower food concentrations.

(iii) Intraspecific interaction stress: Interaction stress was introduced to model excess mortality in the medium and low food supply experiments, where competition for food occurred. Interaction stress was assumed to be absent in the high-food setup but present in the medium and low food setups.

(iv) Emergence stress: Finally, the emergence stress was fitted to minimize the residuals at day 29 with the same stress level for each individual concentration across the high, medium and low food experiments.

All four stress components were added to compose the total stress and converted to modelled survival on a daily basis. Based on the decomposed stress components, in the next step a model was built to predict stress and mortality for any given concentration and day. This model was created with R (version 4.3.1, R Core Team, 2023). SAM and $EC_{x\text{-}SyS}$ modelling was performed using the stress addition package (version 3.1.0). Stress components were fitted using the optimization function optim (package stats, version 3.6.2) with RMSE to be minimized and the lm function (package stats, version 3.6.2) for linear regressions. All figures were created using the package ggplot2 (version 3.4.2).

3. Results and discussion

3.1. Characteristics of the concentration-response relationship

Following one-hour contamination with the pyrethroid esfenvalerate in the mayfly *Cloeon dipterum*, a monotonic log-logistic dose-response relationship was observed within the first 4 days after contamination, regardless of food quantity (see Fig. 1). The corresponding acute LC_{50} was approximately 10 μg/L for all three setups. This aligns with the acute effects observed after one-hour contamination with the analogous pyrethroid fenvalerate in the caddisfly *Limnephilus lunatus* and the crustacean *Gammarus pulex* (Liess, 1994).

Between day 4 and day 8–15, an increased mortality rate was noted at high concentrations (1–100 μg/L) and, to a lesser extent, at ultra-low concentrations (0.001–0.01 μg/L), which are 1000 to 100 times below the acute LC_{50}. Interestingly, at 0.1 μg/L, a hormetic increase in survival was observed compared to both higher and lower concentrations. This tri-phasic concentration-response relationship extends from the two-phasic hormetic relationship initially identified by Schulz (Schulz, 1888) and extensively documented since (Agathokleous et al., 2022b; Calabrese et al., 1999). Notably, the tri-phasic effect relationship shows

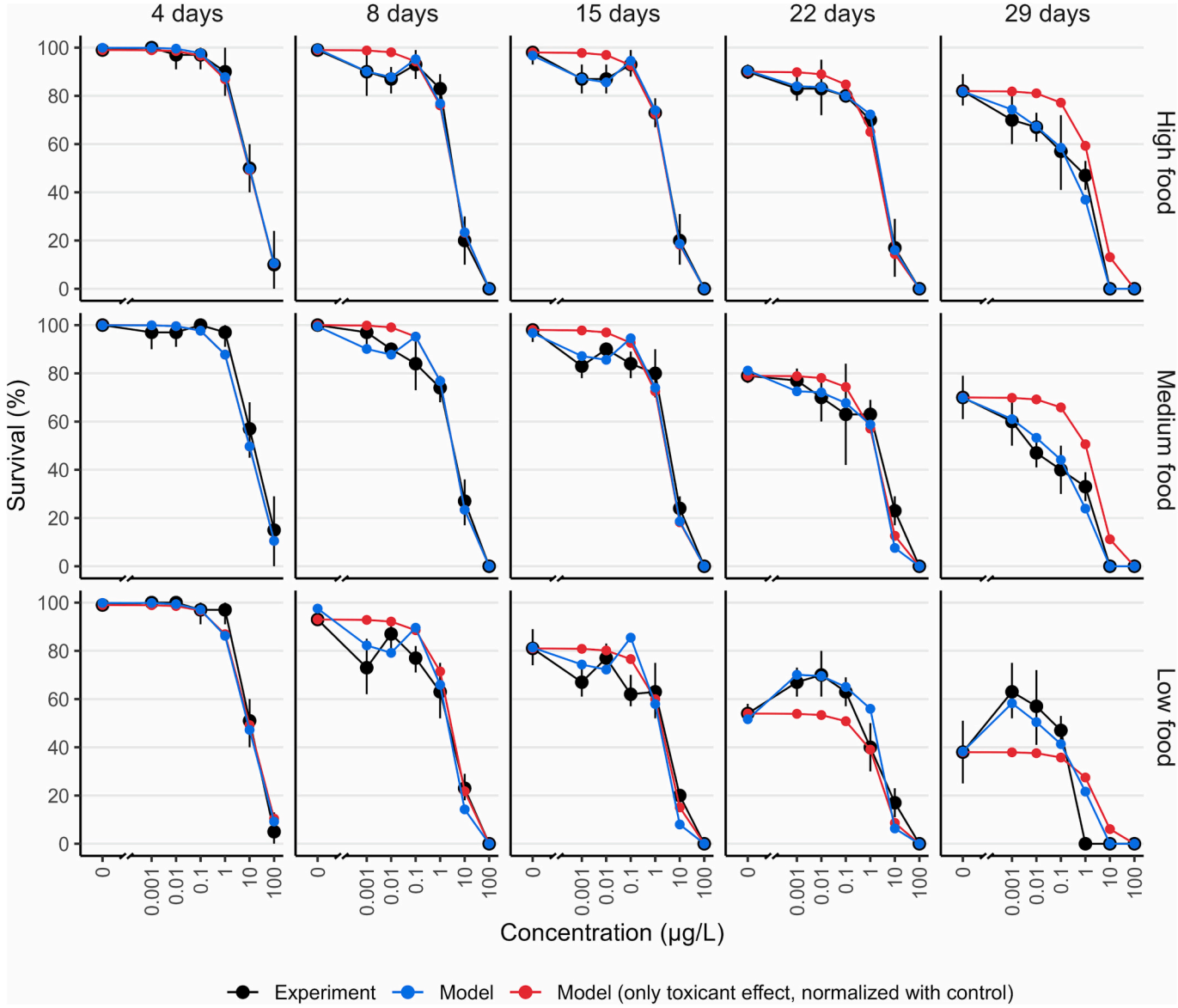

Fig. 1. Observation and modelling of concentration response curves under different food/competition stress. Black dots: Measured survival data from Beketov and Liess (2005); Blue dots: Modelled survival data; Red dots: survival data modelled only with direct pesticide stress including emergence stress but without System-Stress and interaction stress.

a reduced survival at sub-hormetic concentrations and becomes apparent when investigating extremely low concentrations (Liess et al., 2019, 2020). In this initial phase of the experiment, the tri-phasic effect relationship is similar in both the high and low food settings. Mechanistically, the hormetic increase in survival at low concentrations (0.1 µg/L) was attributed to a pesticide-induced reduction in System-Stress (SyS), while the sub-hormetic reduction in survival at ultra-low concentrations resulted from the synergistic interaction between pesticide stress and SyS (Liess et al., 2019).

Between the 22nd and 29th day, a fundamental difference emerged between setups with and without food. In the high food setup, a monotonic concentration-response relationship developed, characterized by decreased survival rates across all concentrations. The hormetic effect observed at 0.1 µg/L disappeared (see Fig. 1A). Conversely, in the low food setup, the survival rate of the control group notably declined during this period, while at low concentrations (0.1–0.001 µg/L), the survival rate remained high. Consequently, in the low food setup and at low concentrations, a population level hormetic increase in the number of survivors was observed from day 22 to 29 (see Fig. 1C). A similar latent increase in survival resulting from pulsed esfenvalerate exposure under food restriction and high intraspecific interactions within the population was recently observed in *Daphnia magna* (Schunck and Liess, 2023).

Only by taking into account the processes of pesticide-induced changes in System-Stress (SyS) and intraspecific competition it is possible to depict a three-phase impact relationship. We show how the SyS causes individual hormesis as well as sub-hormesis; and intraspecific competition causes population hormesis (Fig. 1, blue dots). If only the direct acute pesticide effects and the direct latent effects on emergence are taken into account, only a monotonic effect relationship without ultra-low pesticide effects is depicted (Fig. 1, red dots).

3.2. *Effect strength determines latent effect at the individual level*

The consideration of latent toxic effects enables the quantification of effective concentrations over an extended lifespan. Historically, exposure time has been considered alongside pollutant concentration using Haber's rule to quantify the effect (Haber, 1924). To account for the

exponential influence of time on the latent effect, we used the equation proposed by Druckrey and Küpfmüller (Eq. (5)) (Druckrey and Küpfmüller, 1948). Using this approach, we quantified latent effects at the individual level, in the absence of intraspecific competition (see Fig. 1A), focusing on the "high food" setup, which eliminates the confounding effects of intraspecific competition at the population level.

$$c \times t^n = const. \tag{5}$$

Relationship between the concentration of a toxicant and the exponential duration of exposure (Druckrey and Küpfmüller, 1948). With c: concentration of toxicant; t: time; n: slope of relationship, const.: constant effect size for respective combinations of c and t.

The relationship of Druckrey and Küpfmüller was identified in experiments with continuous contamination. We extended Druckrey and Küpfmüller's relationship for short-term contamination with extended observation periods. To emphasize this distinction, we introduced the exponent "n_{LEA}" to represent Latent-Effect-Amplification (LEA), describing the factor by which the acute effect is amplified by the latent effect (see Fig. 2). We resolved the equation by concentration and in double logarithmic form to linearize the relationship between concentration and time:

$$c = const \times t^{-n_{LEA}} \tag{6}$$

$$logc = logconst. - n_{LEA} \times logt \tag{7}$$

$$n_{LEA} = -\frac{logc - logconst.}{logt} \tag{8}$$

With this approach the concentration of the pesticide and the duration of observation time yielded a latency exponent "n_{LEA}" (Eq. (8)) that describes the LEA (Fig. 2). Remarkably, as the effect strength decreased, "n_{LEA}" increased. For instance, the concentration causing 15 % mortality 29 days after a short contamination was 1000 times lower than the concentration causing the same mortality 4 days after contamination (n_{LEA} = 3,39). This demonstrated that the effect depended to a greater extend on the increase of observation time compared to the increase of toxicant concentration, particularly at lower effect levels. In contrast, at higher effect levels of 50 % ""n_{LEA}" was small (1.26), indicating a much smaller latent amplification. At these levels, the concentration causing

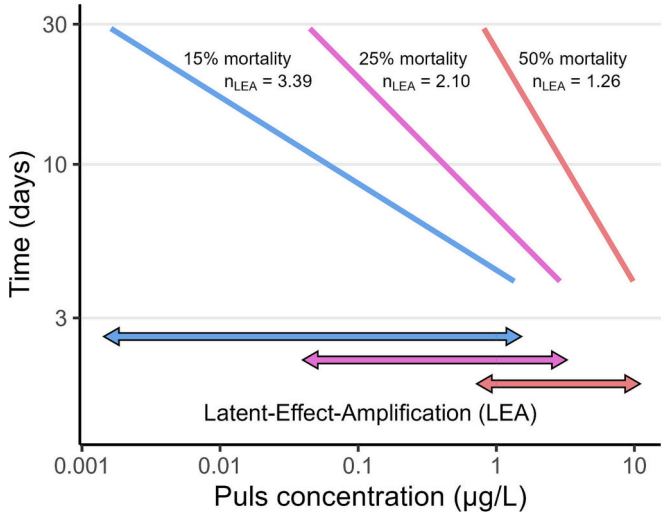

Fig. 2. Iso-effect concentration-time relationships describing the time dependent effect according to Druckrey-Küpfmüller (Eq. (6)). High effects exhibit steeper concentration-time relationships compared to those relationships for lower effects. Here a highly exponential time dependency is present with a greater latency exponent "n_{LEA}". Accordingly, the range of the Latent-Effect-Amplification is larger at low effect levels as compared to high effect levels depicted by the horizontal lines.

50 % mortality 29 days later was only ten times lower than the concentration causing this mortality 4 days after contamination. Additionally, we revealed that over the entire life span, especially at low concentrations, latent effects contributed most to the overall effect. At low concentrations spanning from 1/100 to 1/10,000 of the acute LC_{50}, with a mortality of 30–15 %, latent effects dominated with 92 %–100 % (Fig. 6).

The high contribution of latent effects following pulse contamination challenges the widespread assumption that pyrethroids primarily cause short-term mortality. Two processes may explain these latent effects. Firstly, esfenvalerate's toxicokinetics, owing to its strong sorption in organisms, might result in incomplete degradation or excretion by the experiment's end (Mokkapati et al., 2022). Secondly, the toxicodynamics of esfenvalerate may extend beyond merely interrupting action potentials by binding to open voltage-gated sodium channels, leading to rapid death. These effects also involve largely irreversible changes in receptors, including DNA damage in insects (Saleh et al., 2021) humans (Jurewicz et al., 2015) and disruption of miRNA expression in mouse ovaries, even at the recommended safe dose for humans (Song et al., 2022). Other long-term effects of pyrethroids include largely irreversible effects on insect neurosecretory cells at very low concentrations (Soderlund and Bloomquist, 1989). In essence, at low concentrations, the prevalence of latent effects may be attributed to mostly irreversible alterations in endocrine signalling, affecting individuals through direct receptor interactions, mimicking or synergizing with endogenous hormones, and indirectly impacting signalling pathways (Brander et al., 2016). Thus, pyrethroids like esfenvalerate, and their metabolites, can also be considered endocrine-disrupting chemicals (EDCs) as observed in wildlife (Brander et al., 2016; Marlatt et al., 2022) and human health (Kahn et al., 2020). We conclude that, depending on the concentration, different mechanisms of action may dominate.

3.3. Identifying the latent processes of pesticide effect

To predict the complex concentration-response relationship over time, it is essential to identify the fundamental processes underlying these effects. We investigated the impacts of four stressors and their temporal dynamics to replicate survival patterns across all 21 setups involving 7 concentrations of esfenvalerate and 3 food levels. These stressors included: (i) Direct acute pesticide effects on larvae, (ii) direct latent pesticide effects on adult emergence success, (iii) indirect pesticide effects mediated by System-Stress at the individual level in the absence of intraspecific competition, and (iv) interaction stress at the population level in the presence of intraspecific competition. We used the Stress Addition Model (SAM) to assess the combined effects of these stressors, using "general stress" as a common currency for independent stressors (Liess et al., 2016). With SAM, all stressors synergistically contributed to the overall effect compared to the null model of effect addition (Bliss, 1939). For details on joint stressor calculation, please refer to the methods section.

The contribution of these identified processes is illustrated in Fig. 3 and can be summarized as follows:

(i) Acute pesticide effects – Direct acute pesticide stress on larvae declined exponentially over time, exerting a minor effect, compared to the other stressors, especially at low concentrations (Fig. 3A; Fig. 1, red line).

(ii) Individual level hormesis and tri-phasic response relationship – Pesticide exposure, even at the lowest concentration, initially reduced System-Stress (SyS) and may be considered as a compensation process at the individual level (SyS) (Liess et al., 2019). Then, at low concentrations a rebound of SyS was observed (Fig. 3B) occurring progressively later with increasing concentration (Fig. 4B), as also described in Liess et al., 2019 (Liess et al., 2019). This change in SyS causes the temporal dynamics of hormesis at the individual level and the tri-phasic

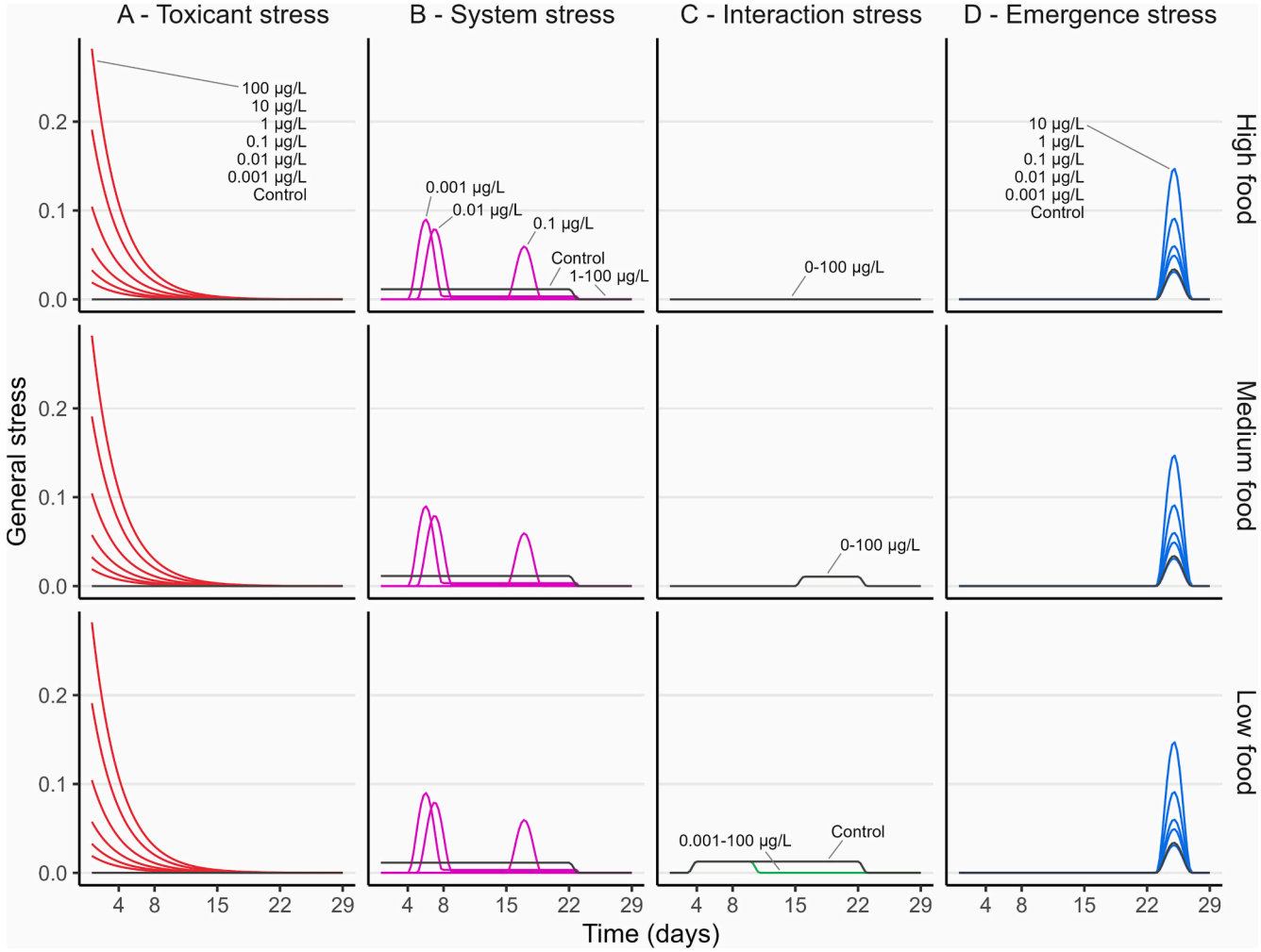

Fig. 3. Temporal progression of the development of relevant stress components extracted from the experimental observation as detailed in the method section. A – Toxicant stress reduction over time; B – System-Stress (SyS) including rebound. See also Fig. 4A; C – Interaction stress; D – emergence stress, no emergence at 100 µg/L Esfenvalerate.

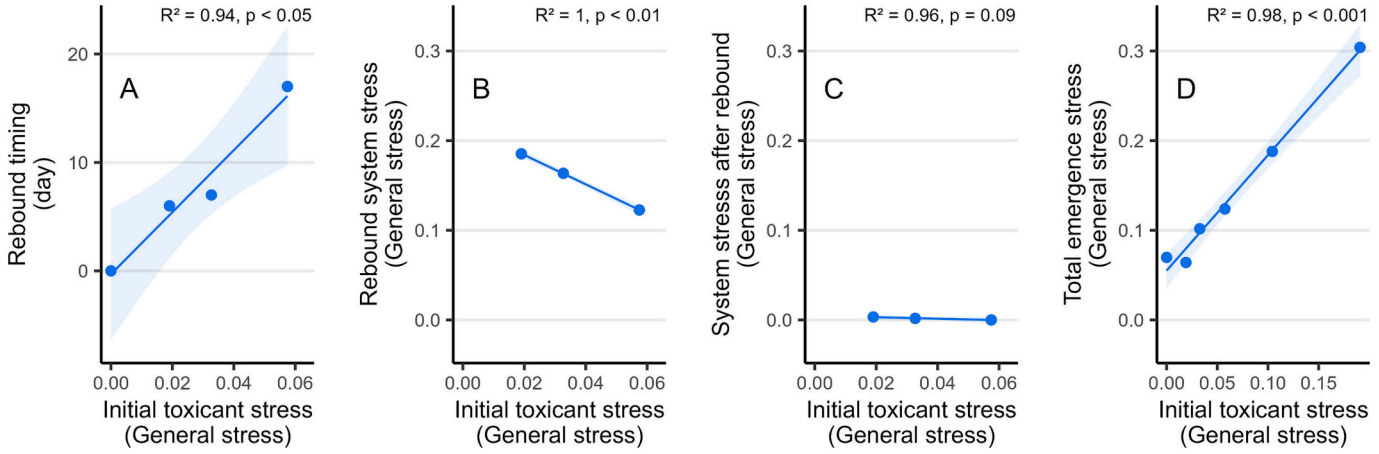

Fig. 4. Latent pesticide effects and their relation to initial toxicant stress. A) Latency of rebound timing as displayed in Fig. 3 B); Strength of rebound of System-Stress (SyS). C) Strength of SyS after the rebound; D) Magnitude of emergence stress.

response relationship. Also in pharmacology, rebound processes are known as "discontinuation effect", in which the withdrawal of a drug causes an exaggerated reaction. Substances where such processes have been observed include: Heparin, HMG-CoA Reductase Inhibitors, Calcium Channel Blockers, α-Adrenergic Receptor Antagonists (Reidenberg, 2011). The physiological basis of the reduced SyS induced by pesticides may lie in the reduced metabolic demands through changes in behaviour,

physiology and cellular biochemistry, all of which contribute to lowering whole organism energy expenditure (Richards, 2010). This pattern appears common under stress exposure, as evidenced by fine sediment deposition, increased salinity, and reduced flow velocity, which led to the strong transcriptional suppression of genes involved in metabolic and energy-consuming cellular processes (Brasseur et al., 2022) and may be regarded as a compensatory reaction. Mechanistically, the temporary decrease in SyS leads to temporary hormesis, while the rebound in SyS initiates the sub-hormesis of the tri-phasic response relationship. Accordingly, individual hormesis occurs within concentration ranges where SyS is reduced, and direct negative pesticide effects are minor, while sub-hormesis occurs at lower concentrations where rebound has already occurred interacting synergistically with toxicant stress, leading to increased mortality. An individual level related hormesis.

(iii) Population level hormesis – Strong intraspecific competition was observed, especially at the controls of the low food setups (see Figs. 3C and 4C). Interestingly, even the lowest esfenvalerate concentration led to a decrease in interaction stress after 10 days, at low food levels, even with a constant population density (see Fig. 3C). This reduction in intraspecific competition may be considered as a compensation process at the population level causing the development of hormesis at the population level. A comparable reduction in intraspecific interaction as a result of ultra-low pesticide exposure and followed by an increased abundance was recently observed in populations of *Daphnia magna* (Schunck and Liess, 2023). Consequently, starting from day 22, at low concentrations (0.001 μg/L – 0.1 μg/L), the population with the highest intraspecific competition exhibited a hormetically improved survival rate compared to the control. Accordingly, this hormetic response relates to a population level hormesis and is distinguished from an individual level hormesis as described above in (ii).

(iv) Latent effects on emergence – During emergence, stress and associated mortality increased with rising acute pesticide stress more than three weeks after the one-hour exposure (see Fig. 3D - Emergence stress). Emergence stress is tightly linked to the pesticide stress (Fig. 4D). It appears that the process of emergence is particularly susceptible to the effects of pyrethroids, consistent with previous investigations (Rogers et al., 2016). Thus, it is apparent that the latent reduction of emergence may contribute decisively to the frequently described reduction of insect biodiversity by pesticides.

The individual stress components presented in Fig. 3 were interpolated as shown in Fig. 4 and were cumulatively assessed on a daily basis using SAM. The resulting modelling of the concentration-response relationship is illustrated in Fig. 5. From this, the contribution of latent effect to total mortality is depicted for the high food set-up (Fig. 6). In summary, this analysis reveals that latent effects result from the synergistic interaction of SyS rebound, pesticide stress, and emergence stress following pesticide exposure. Furthermore, compensatory processes lead to a temporary hormetic increase in the survival rate at both the individual and population levels.

3.4. Implications for regulatory effect assessment

When assessing the effects of pesticides and other toxic substances, LC_{50} values or similarly high effect levels are generally used as the basis for toxicity assessment. This approach also extends to the estimation of concentrations with low effects like LC_5 or LC_{10}, as these measures are typically extrapolated from high effect concentrations using a monotonic dose-response relationship. We posit that this approach presents a fundamental problem, particularly because the relevance of latent effects becomes significantly more pronounced at low effect sizes compared to high effect sizes. Extrapolating low effect sizes from high effect sizes, while assuming a monotonic response, inadequately accounts for latent effects. Indeed, findings from field studies indicate that

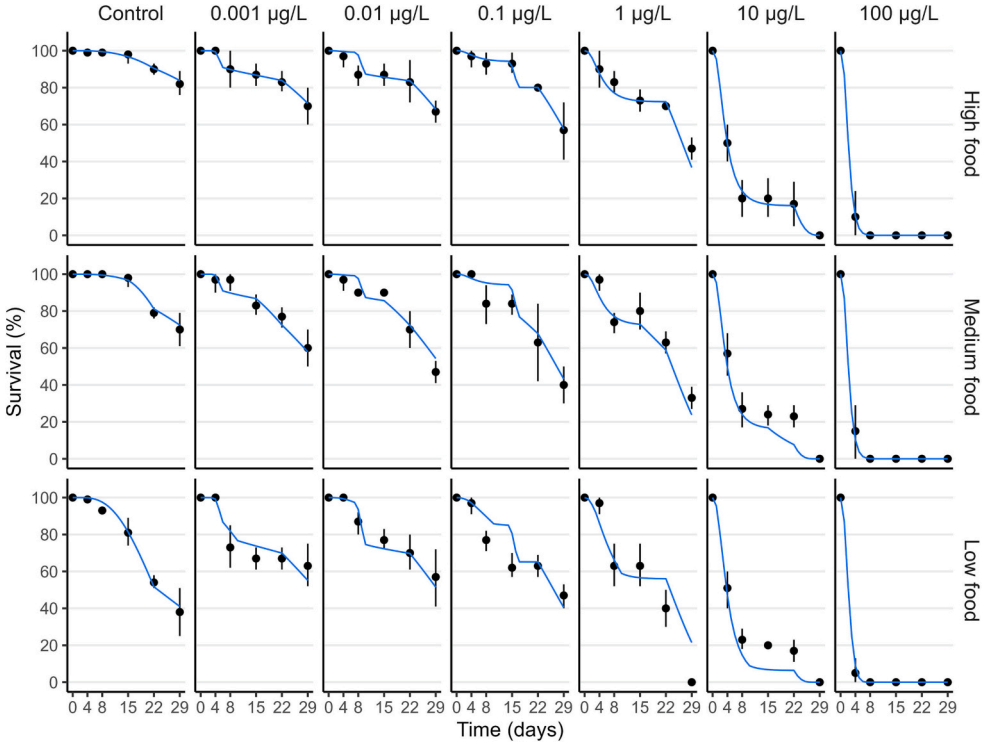

Fig. 5. Modelling of abundance on the basis of the Stressor Addition Model (SAM) as a function of esfenvalerate concentration and food quantity. Black dots: Measured survival data from Beketov and Liess (2005); Lines: Modelled survival based on the SAM framework.

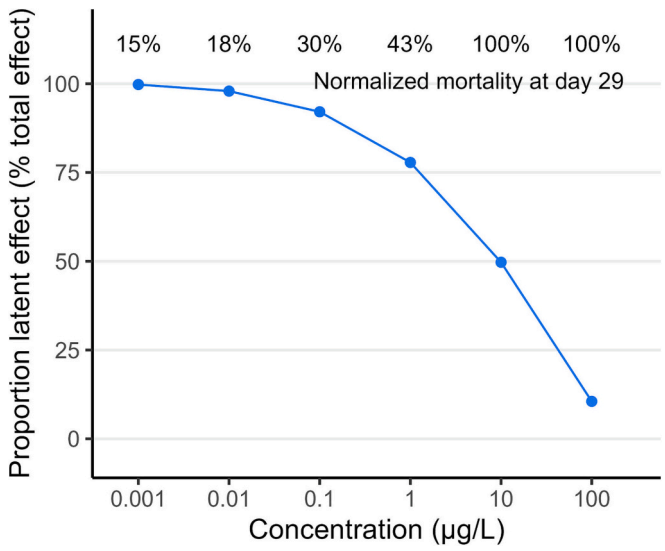

Fig. 6. Contribution of latent effect to total mortality. Expressed as mortality after day 4 in relation to mortality in the first 4 days. Mortality normalised with control. Numbers on top relate to cumulative mortality on day 29 (modelled).

existing legal thresholds often fail to protect ecosystems adequately. For instance, substantial impacts on vulnerable insects in agricultural streams were observed even when exposure levels were four orders of magnitude below the acute LC_{50} (Liess et al., 2021).

In our present study, we demonstrate that even at concentrations four orders of magnitude below the acute LC50 (0.001 µg/L), there was a pesticide-related mortality of 15 %. Such low-effect levels may lead to population extinction when interspecific competition with a less vulnerable species is present, and effects of repeated contamination events culminate (Liess et al., 2013). Conversely, in the absence of interspecific competition, a strong toxicant effect can result in population recovery. Future risk assessments should therefore consider both the environmental context and the magnitude of the effect to establish safe thresholds that prevent adverse impacts on populations.

The hormesis phenomenon observed here, resulting in a tri-phasic dose-response relationship, is not consistently integrated into the official risk assessments conducted by organizations such as EFSA, US-EPA, and China's Ministry of Ecology and Environment (Agathokleous et al., 2022a). Consequently, predicting and extrapolating high effect levels generated in laboratory test systems to ecologically relevant low effect concentrations introduce additional uncertainty. For example, in pesticide risk assessment within aquatic environments, it is generally assumed that 1/100 of the acute LC50 provides protective thresholds for field effects (EFSA, 2013). However, in the study presented here, hormetic compensation processes reduced mortality to near-control levels for up to 15 days at 1/100 of the acute LC50 of Esfenvalerate, particularly under high-food conditions (Fig. 5). Only after this time period did the latent effect of the pesticide start to reduce survival by 30 % compared to the control.

In summary, it is evident that conventional effect assessment extrapolation approaches do not offer adequate protection. Safety factors should be increased to consider latent effects appropriately. This underscores the need to extend existing approaches, as it is not just the dose that makes the poison (Paracelsus), but the Latent-Effect-Amplification that truly defines the toxicity of substances.

CRediT authorship contribution statement

Matthias Liess: Conceptualization, Guiding analytical cognition process, original draft. Jonas Gröning: Data curation, Formal analysis, Visualization.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

Data are available within the R-Script and the associated data file within the supplementary materials

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168368.

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