



# One like all? Behavioral response range of native and invasive amphipods to neonicotinoid exposure<sup>☆</sup>

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## ABSTRACT

Native and invasive species often occupy similar ecological niches and environments where they face comparable risks from chemical exposure. Sometimes, invasive species are phylogenetically related to native species, e.g. they may come from the same family and have potentially similar sensitivities to environmental stressors due to phylogenetic conservatism and ecological similarity. However, empirical studies that aim to understand the nuanced impacts of chemicals on the full range of closely related species are rare, yet they would help to comprehend patterns of current biodiversity loss and species turnover. Behavioral sublethal endpoints are of increasing ecotoxicological interest. Therefore, we investigated behavioral responses (i.e., change in movement behavior) of the four dominant amphipod species in the Rhine-Main area (central Germany) when exposed to the neonicotinoid thiacloprid. Moreover, beyond species-specific behavioral responses, ecological interactions (e.g. parasitism with Acanthocephala) play a crucial role in shaping behavior, and we have considered these infections in our analysis. Our findings revealed distinct baseline behaviors and species-specific responses to thiacloprid exposure. Notably, *Gammarus fossarum* exhibited biphasic behavioral changes with hyperactivity at low concentrations that decreased at higher concentrations. Whereas *Gammarus pulex*, *Gammarus roeselii* and the invasive species *Dikerogammarus villosus*, showed no or weaker behavioral responses. This may partly explain why *G. fossarum* disappears in chemically polluted regions while the other species persist there to a certain degree. But it also shows that potential pre-exposure in the habitat may influence behavioral responses of the other amphipod species, because habituation occurs, and potential hyperactivity would be harmful to individuals in the habitat. The observed responses were further influenced by acanthocephalan parasites, which altered baseline behavior in *G. roeselii* and enhanced the behavioral response to thiacloprid exposure. Our results underscore the intricate and diverse nature of responses among closely related amphipod species, highlighting their unique vulnerabilities in anthropogenically impacted freshwater ecosystems.

## 1. Introduction

Detrimental shifts in biodiversity have become increasingly evident in recent decades (Bernhardt et al., 2017; Butchart et al., 2010; Jaur-eguiberry et al., 2022), including in freshwater ecosystems (Albert et al., 2021; Baranov et al., 2020; Reid et al., 2019) even if several larger rivers

in Europe show other, more positive population trends (Haase et al., 2023; Nguyen et al., 2023). These positive trends can be tracked back to successful efforts to improve the quality of these systems, after a pollution peak in the 1980s. In recent years, however, stagnation has been observed, which presumably result from the introduction of new chemicals (Haase et al., 2023). Nevertheless, our understanding of the

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relationship between chemical pollution and its consequences on the composition of aquatic species communities and the broader loss of biodiversity remains incomplete. The intricate dynamics of how chemicals interact with ecosystems, influencing species interactions, population dynamics, and overall ecological health, is still poorly understood (Sigmund et al., 2023; Sylvester et al., 2023).

Environmental risk assessment of chemicals typically involves a series of standardized tests to understand and evaluate the potential impacts on ecosystems. The process often includes toxicity testing with few species as representatives of a complex biocenosis, estimation of environmental exposure pathways, and consideration of factors like bioaccumulation and persistence. The assessment usually employs standardized ecotoxicological test guidelines (OECD, ISO norm) with test organisms representing different trophic levels (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013). Toxicological parameters (e.g., EC<sub>50</sub>, NOEC or EC<sub>10</sub> values) are derived from these standard tests and divided by safety or assessment factors to allow extrapolation within and between species, from laboratory to field conditions and from a few standard organisms to the whole biocenosis to obtain safe concentrations for the ecosystem (Brack et al., 2019; Neale et al., 2023; Vighi & Villa, 2013). Approaches like these have made a significant contribution towards demonstrating toxic relationships and reducing environmental pollution. However, these approaches often use a limited number of test species, potentially providing an incomplete picture of the effects on locally relevant species (Breitholtz et al., 2006). A narrow range of test species may not fully capture the diverse responses exhibited by different individuals in a natural ecosystem, with some being more sensitive to a particular chemical and others more resilient.

A compelling example comes from the silver salmon (*Oncorhynchus kisutch*) which is highly sensitive to 6PPD-quinone (LC<sub>50</sub> = 0.095 µg/L), a transformation product originating from rubber tire material (Tian et al., 2021). A study by Brinkmann et al. (2022) showed that among three closely related species tested, only two demonstrated this heightened sensitivity, namely the brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*), while the Arctic charr (*Salvelinus alpinus*) did not display a comparable level of sensitivity. With regard to invertebrates, examples in *Daphnia* spp. have shown enhanced sensitivity of non-standard species compared to *D. magna* towards veterinary antibacterials (Dalla Bona et al., 2014), heavy metals and organics (Cui et al., 2018; Santos-Medrano & Rico-Martinez, 2019).

To expand and improve environmental risk assessments, there is a growing recognition of the need for more comprehensive and ecologically relevant testing methodologies (e.g., non-standard-studies). These studies often involve more realistic conditions, a broader range of species that better represent the diversity of local ecosystems, and often field observations to provide a comprehensive understanding of the potential ecological impacts of chemicals (Ågerstrand et al., 2020; Bertram et al., 2022; Ford et al., 2021; Vighi & Villa, 2013). This addresses, for example, the shortcoming that standard test species such as *D. magna* might not be suitable to represent locally relevant riverine invertebrates. For example, freshwater amphipods of the genus *Gammarus* hold important ecosystem functions and are known to be more sensitive towards organic pollutants than *D. magna* (Von der Ohe & Liess, 2004). Amphipods are not only commonly found in most central European riverine ecosystems, often in high abundances, but are also considered keystone species in many riverine ecosystems due to their critical role in nutrient cycling and serving as a primary food source for higher trophic levels, such as fish, birds, and other macroinvertebrates (MacNeil et al., 1997, 1999). Additionally, amphipods are also intermediate hosts for acanthocephalan parasites, which further shape their behavior and potentially impact ecosystem health (Giari et al., 2020; Kuhn et al., 2015; Lefèvre et al., 2009). Interaction with the acanthocephalan parasite can influence the survival of gammarids when exposed to chemicals, possibly because the metabolism of pollutants is affected or the accumulation of pollutants in the organism is altered (Kochmann

et al., 2023). Overall, amphipod communities are under diverse anthropogenic pressure and experience a turnover from native to invasive species in the big rivers. The most recent example is the immigration of the so-called killer shrimp *Dikerogammarus villosus*, which nowadays belongs to the dominant amphipod species in the Rhine system (Leuven et al., 2009; Soto et al., 2023; Van Riel et al., 2011). Native species such as *G. pulex* and *G. fossarum* continue to dominate in the tributaries, along with *G. roeselii*, which is considered to be naturalized (Grabowski, 2007). Turnover in the amphipod community is caused not only by invasive species, but also by pollutant discharges. *G. fossarum* – a species that is mainly found in the upstream areas of rivers – usually emerges as the loser (Enns et al., 2023). These are indications that tolerances may not necessarily be phylogenetically conserved, but that despite close relatedness (Copilaş-Ciocianu et al., 2020) and high morphological and ecological similarity (Jourdan et al., 2016; Pelikan et al., 2024; Pöckl, 1993), species-specific processes are the cause of the vulnerability within the locally abundant amphipods.

In the present study, we investigate the response of four widespread and ecologically relevant amphipod species to the neonicotinoid insecticide thiacloprid. We chose thiacloprid as an important representative of the neonicotinoids. Thiacloprid has been found in concentrations which exceeded regulatory acceptable concentrations by Betz-Koch et al. (2023) in the same study area as we collected our test species from as well as in comparable freshwaters in Germany (Halbach et al., 2021). In order to capture the unique responses of each species, we follow the call to prioritize sublethal endpoints and have thus measured the behavioral responses (Ågerstrand et al., 2020; Ford et al., 2021) of these organisms as a sublethal endpoint. To accomplish this, we used the recently established behavioral monitoring tool ToxMate (Soose et al., 2023). With ToxMate and comparable systems, changes in behavior can be detected at low environmentally relevant concentrations (De Lange et al., 2006; Lebrun et al., 2023; Soose et al., 2023). This is beneficial, because behavioral changes hold significant ecological relevance, impacting individual fitness, population dynamics, species interactions and ecosystem functions (Saaristo et al., 2018; Candolin & Wong, 2019). This underscores the importance of behavioral ecotoxicology, as alterations in behavior can trigger chain reactions affecting multiple trophic levels and disrupting natural ecosystems (Bertram et al., 2022; Bundschuh et al., 2020; Ford et al., 2021). In this context it is important to note that acanthocephalan parasites represent a natural stressor that has been shown to interact with their amphipod hosts and influence their behavior (Bauer et al., 2000, 2005). Acanthocephala are endoparasites that use aquatic invertebrates as intermediate hosts. The manipulated behavior increases the predation risk for the amphipod and thus the probability for the parasite to enter the final host (Bauer et al., 2000, 2005; Lefèvre et al., 2009). These behavioral changes are mostly investigated in species that coevolved over a longer time, such as *G. pulex* (Bauer et al., 2005). Recent observations of prevalences of up to 73% and a potential positive effect of acanthocephalan parasites on intermediate hosts under pyrethroid exposure resilience of *G. roeselii* (Kochmann et al., 2023) have prompted us to also consider this interaction in our study.

In our behavioral ecotoxicology approach, we first expected slight ecological differences to be reflected in different baseline behavior, for example, the invasive species *D. villosus* described as sit-and-wait predator should exhibit lower activity, contrasting with the ecologically more similar gammarids (*G. pulex* and *G. fossarum*) that were expected to display comparable baseline behavior. Second, we expected low concentrations of thiacloprid to evoke hyperactivity/increased activity (i.e., a flight response) and a successive decrease in activity with increasing concentrations (i.e., mode of action or physiological regulation processes). More specifically, we hypothesized to find this pattern to be particularly pronounced in *G. fossarum*, for which a negative population trend was shown in response to chemical exposure (Enns et al., 2023). In contrast, species like *D. villosus* and *G. roeselii*, known to benefit from anthropogenic impacts (Enns et al., 2023; Grabowski,

2007; Rewicz et al., 2014), were anticipated to display behavioral changes only at higher concentrations of thiacloprid. Thirdly, we expected that the interaction with acanthocephalan parasites would lead to increased activity.

## 2. Material and methods

### 2.1. Study species and sampling sites

In our study, we considered the four most common amphipod species in the Rhine-Main metropolitan region (central Germany). The species differ in their invasion status (native, naturalized, or invasive) and often inhabit different sections of the stream. However, they do share certain similarities, such as their omnivorous and predatory dietary habits (MacNeil et al., 1999; Piscart et al., 2011), as well as similar habitat preferences (Devin et al., 2003; Kley & Maier, 2005). These shared traits, coupled with the frequently observed pronounced intraguild predation by *D. villosus* (MacNeil & Platvoet, 2005), intensifies the competition among these species. Consequently, this competitive pressure often results in the displacement of native species by *D. villosus* in numerous river ecosystems. According to Hou & Sket (2016), all four of our test species are classified within the Gammaridae family. Specifically, *G. fossarum*, *G. pulex*, *G. roeselii* exhibit a closer phylogenetic relationship to each other than to *D. villosus* (Hou et al., 2007).

*G. fossarum* (Koch, 1835) occurs most upstream in the sampling area and has high sensitivity towards anthropogenic contaminants (Enns et al., 2023; Meijering, 1991). Another native species is *G. pulex* (Linné, 1758) which can be found in middle to low areas of streams (Karaman & Pinkster, 1977; Janetzky, 1994), where it is usually in contact with anthropogenic pollutants (Soose et al., 2023). *G. roeselii* (Gervais, 1835) has its main distribution area on the Balkan Peninsula. The genetic lineage C has further spread northwards and westwards outside the Danube system in recent centuries (Csapó et al., 2020). Due to its ability to coexist with native amphipod species, it is frequently considered a naturalized species in river basins north and west of the Danube catchment area (Grabowski, 2007; Csapó et al., 2020; Josens et al., 2005). *G. roeselii* often shows a high adaptive capacity and still occurs even under certain anthropogenic pressure in middle and low stream sections where the two native gammarids (*G. fossarum*, *G. pulex*) disappear (Karaman & Pinkster, 1977; Jourdan et al., 2019, 2024). The invasive species *D. villosus* (Sowinsky, 1874) originates in the Ponto-Caspian region from where it began to successfully invade central and western Europe in the 1990s (Bij de Vaate et al., 2002; Grabowski, 2007; Podwysocki et al., 2024). *D. villosus* are often attributed a particularly predatory behavior with a sit-and-wait predatory strategy (Maazouzi et al., 2011; Platvoet et al., 2009).

All these amphipods can serve as intermediate host for acanthocephalan parasites. In Europe, *Pomphorhynchus laevis*, *P. tereticollis*, and *Polymorphus minutus* appear to be the most prevalent aquatic Acanthocephala (Schmidt-Rhaesa, 2015). The role of these infections in the face of chemical pollution is recently discussed as potentially advantageous, as ecotoxicological studies have observed higher survival rates of infected amphipods in the presence of chemical pollution (Grabner et al., 2023; Kochmann et al., 2023). Kochmann et al. (2023) further observed exceptional high prevalences (up to 73% mainly *P. laevis*) of *G. roeselii* in a polluted river.

We sampled the test individuals via multi-habitat sampling at the different sites using hand nets (Bioform V2A; mesh size 500 µm, bioform Dr. J. Schmidl e.K., Nürnberg, Germany). *G. fossarum* was collected in the near pristine headwaters of the Urselbach (50.216959, 8.530449). *G. pulex* was sampled in the lower reaches of the same stream, approximately 1.5 km downstream the effluent of a wastewater treatment plant (50.168744, 8.621132). We collected *G. roeselii* at the Horloff River – a massively anthropogenically impacted river in the Main catchment – where we re-sampled a population that has already been studied several times before (50.379902, 8.887866; i.e., sampling site 5 according to

Kochmann et al., 2023; Jourdan et al., 2024). Invasive *D. villosus* were collected at the river Main (50.080406, 8.522079) by gently removing the individuals from the bottom of bigger stones and rocks, where they hide between small freshwater mussels of the genus *Dreissena*. After collecting the individuals, we immediately transferred them to aerated cooling boxes (30 L) containing stream water from the sampling site and brought them to our laboratory.

### 2.2. Molecular species identification

Since most of our study species actually represent – often unconsidered – cryptic species complexes (Jourdan et al., 2023), we genetically characterized our test populations using DNA barcoding. For the cryptic species complex *G. roeselii* only MOTU C occurs in Germany (Csapó et al., 2020). Invasive *D. villosus* in the Rhine-Main system originate from the western invasions route and also belong to only one lineage (Rewicz et al., 2015). We have therefore limited the genetic species identification to the cryptic *G. pulex* and *G. fossarum*. For this purpose, we removed 2–3 pleopods from 7 to 8 individuals per population using sterile forceps. We then extracted the DNA from each specimen using the EZNA tissue DNA kit (Omega bio-tek, Norcross, Georgia, USA) according to the manufacturer's protocol. The cytochrome c oxidase subunit I (COI) gene fragment was amplified using a pair of universal primers LCO1490 and HC02198 according to the recommendations of Folmer et al. (1994). For each PCR reaction, 2 µL of template DNA was combined with a mixture comprising 7.1 µL H<sub>2</sub>O, 0.2 µL Bovine Serum Albumin (BSA), 1.8 µL MgCl<sub>2</sub>, 1.5 µL Taq buffer, 1.2 µL dNTPs and 0.2 µL Taq polymerase. Additionally, 0.5 µL of the forward primer (LCO1490) and 0.5 µL of the reverse primer (HC02198) were added to the reaction mix. PCR amplification was conducted using the following thermal cycling conditions on thermal cycler (Arctic Thermal Cycler; Thermo Fisher Scientific, Vantaa, Finland): initial denaturation at 94 °C for 2 min, followed by 34 cycles of denaturation at 94 °C for 20 s, annealing at 46 °C for 30 s, elongation at 65 °C for 60 s, and a final extension at 65 °C for 5 min. PCR products were visualized using agarose gel electrophoresis to confirm successful amplification. Trimming the alignment to the shortest sequence resulted in a 577 bp sequence. The quality-checked sequences were compared to the NCBI nucleotide database via online BLAST search (Basic Local Alignment Search Tool). All *G. fossarum* were identified as *G. fossarum* type B (or clade 11; Wattier et al., 2020; Weigand et al., 2020). All *G. pulex* belong to *G. pulex* clade D (Lagrué et al., 2014; Weigand et al., 2020). The nucleotide sequences of the COI gene fragment were deposited in BOLD (Barcode of Life Database) under the Process IDs TOXM001-24 to TOXM015-24.

### 2.3. Amphipod cultivation in the laboratory

After sampling, the individuals stayed in their original river water for 24 h and were then transferred to 54 L aquaria with a maximum stocking of 200 individuals and slowly acclimatized to laboratory conditions and artificial medium, that met the specific water quality demands of the species. The medium for *G. pulex*, *G. roeselii*, and *D. villosus* consists of 0.125 g mineral salt (Preis-Diskus-Mineralien, Preis-Aquaristik KG, Bayerfeld, Germany), 0.3 g NaHCO<sub>3</sub> (VWR international GmbH, Darmstadt, Germany), and 0.167 g CaCl<sub>2</sub> (Supelco, Merck KGaA, Darmstadt, Germany) per liter of deionized water. The medium for *G. fossarum* contained only 0.15 g mineral salt (Preis-Diskus-Mineralien, Preis-Aquaristik KG, Bayerfeld, Germany) per liter of deionized water. In preliminary tests, these media proved to be the best for long-term maintenance of our test species. The acclimatization lasted at least 7 days before the experiment started. Individuals were kept at 10 °C in a climate-controlled room with a 12:12 h light:dark cycle. Ceramic filter tubes and gravel served as hiding places in the aquariums. Leaf litter (*Alnus glutinosa*, *Fagus sylvatica*) and shrimp flaked food (Shrimp King Mineral, Dennerle, Münchweiler, Germany & TetraMin Flakes, Tetra, Melle, Germany) were fed *ad libitum*.

## 2.4. Experimental setup

The experimental setup was based on the method described in Soose et al. (2023) with the following modifications: For quantification of behavioral changes of the test individuals, we first acclimatized them for 30 min in the ToxMate (also referred to as ToxmateLab; Viewpoint, Lyon, France) before exposing them for 120 min to thiacloprid. Artificial medium described in 2.3. was used to perform the experiments. With help of a pre-tempered medium and an external cooling device (RC-10 Digital Chiller, VWR, Darmstadt, Germany), we established a temperature of  $14 \pm 1$  °C during all experiments. The mean dissolved oxygen level in the experimental medium was 10.5 mg/L before the experiments and 9.88 mg/L after. All experiments were consistently conducted at approximately 9 a.m. local time, with consistent lighting conditions (set to 500 lux) and on consecutive days to mitigate potential effects from the diurnal rhythm of our test individuals. The timebins used for summarizing the swimming behavior were 300 s, while every 60 s the background was refreshed (ToxMate software settings). The grayscale setting of 10 was set to efficiently track the amphipods. The sum of movement (total distance moved [m] (TDM)) and the time spent active [%] (TA) per individual was used in our final dataset. Per treatment (e.g., negative control, exposure concentrations) a total number of 32 randomly chosen individuals were used in the two vertically oriented panels of the ToxMate. The panels consist of polymethylmethacrylate, polyethylene and thermoplastic elastomers with biocompatibility qualification (FDA and USPC-6). The test chambers for each individual measures  $55 \times 50 \times 18$  mm (Soose et al., 2023). Each test series contained a negative control and five concentrations for each test species. Immediately after each test run, the individuals were individually fixed in 96% ethanol to determine their sex and size. New individuals were used for each run. Individuals were sexed according to external sexual characteristics indicating sexual maturity of test individuals. The body length [mm] of each individual was measured from the tip of the rostrum to the telson tip (see Jourdan et al., 2019 for details). For this study we individually tracked the behavior of 958 individuals of four local amphipod species. The overall sex-ratio (male:female) was nearly balanced with 1.2:1 (for further details see Supplementary Table S 1).

## 2.5. Test substance

Thiacloprid (CAS 111988-49-9) belongs to the group of neonicotinoids. Thiacloprid has a high water solubility of 185 mg/L (at 20 °C) and a low log  $K_{OW}$  of between 0.76 and 1.26 (at 20 °C) (European Commission, 2008; Sigma Aldrich, 2023). Thiacloprid is detected in the freshwater environment with a maximum measured environmental concentration (MEC) of 4.69 µg/L (IPChem Portal, 2022). In our study area, the Rhine-Main metropolitan region, thiacloprid concentrations of 0.7 µg/L were measured during heavy rainfall events between 2017 and 2018 (Betz-Koch et al., 2023). Thiacloprid is neurotoxic by acting as an agonist at the nicotinic acetylcholine receptors resulting in permanent transmission of stimuli through the postsynaptic cell (Tomizawa & Casida, 2005; Matsuda et al., 2020). It is relatively stable towards hydrolysis under various pH values (Chen et al., 2021). Information on 50% dissipation time (DT<sub>50</sub>) varies between 10 and 63 d according to the medium in which the substance is dissolved (U.S. EPA, 2003; Chen et al., 2021). In 2020, the registration for usage of thiacloprid in the European Union was discontinued and the remaining products had to be utilised by February 03, 2021 (European Union, 2023).

Sublethal concentrations are regarded as 10% of the LC<sub>50</sub> value or less (Amiard-Triquet, 2015; Little & Finger, 1990). Our second highest concentration was about 10% of the LC<sub>50</sub> (101 µg/L) of the most sensitive population of *G. roeselii* across a pollution gradient (Jourdan et al., 2024). The exposure design comprised five concentrations (0.32, 1.04, 3.31, 10.6, 33.92 µg/L) and the negative control. Thiacloprid PESTANAL®, was purchased from Sigma-Aldrich (Taufkirchen, Germany) with a minimal purity of 98%. Concentrations were prepared as a dilution

series with the spacing factor of 3.2. We prepared fresh solutions for each test series. Actual exposure concentrations were checked by analysing three samples of the highest levels using liquid chromatography coupled to high resolution mass spectrometry. To this end, 5 µL of the exposure media were directly injected for LC separation, detected after electrospray ionisation in positive mode and quantified by internal standard calibration using imidacloprid-D4. The limit of quantification was 30 ng/L. The average recovery in these samples was  $116 \pm 9$  % of the nominal concentrations.

## 2.6. Relevance and reliability of the method

Enhancing the applicability and credibility of non-standard studies in risk assessment calls for a systematic and transparent presentation of ecotoxicological methodologies and findings, as suggested by Ågerstrand et al. (2020) and Ford et al. (2021). Recognizing the importance of standardized reporting, Moermond et al. (2016) introduced a comprehensive checklist specifically tailored for ecotoxicological studies, streamlining the review process. In alignment with this guidance, we have incorporated the completed reporting guidelines checklist for our study into the supplementary material of this paper (Supplementary Table S 4). An extension of the reporting criteria specifically for behavioral studies (EthoCRED) is the subject of the current work by Bertram et al. (under revision). We already mention many of the criteria in our study and they should be applied in future studies.

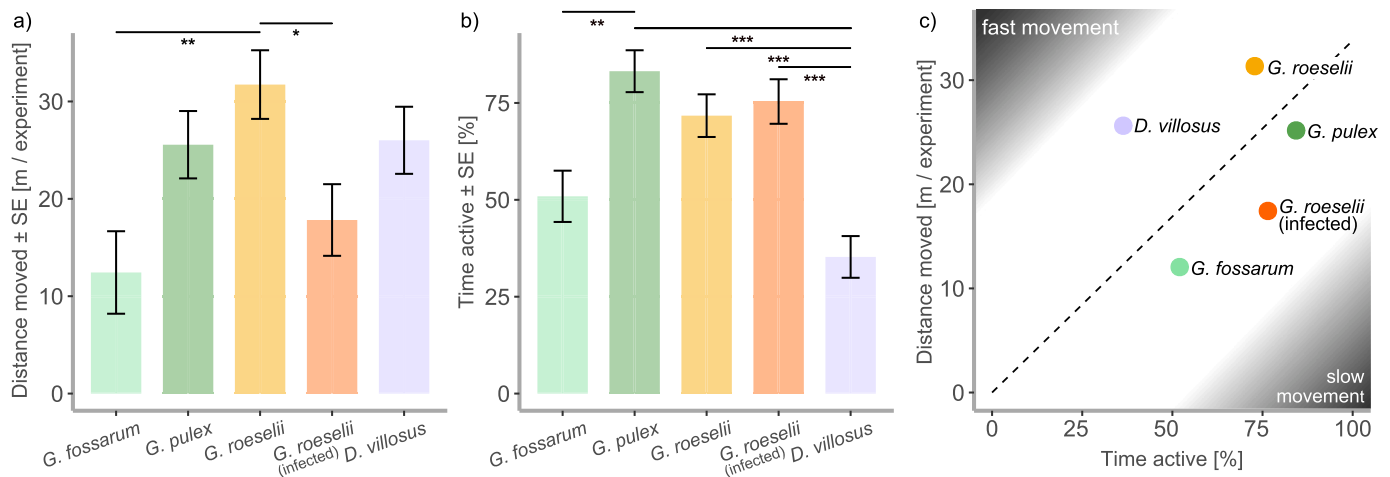
## 2.7. Statistical analyses

We applied linear models using the lm-function implemented in R version 4.1.2. The data sets distance moved 'TDM', and activity 'TA' served as dependent variables in separate linear models. Infected and uninfected *G. roeselii* were tested individually and were coded as different species for statistical analysis (i.e. '*G. roeselii* infected' and '*G. roeselii* uninfected'). The 'species', 'concentration', 'body size', and 'sex' were used as explanatory variables. In the initial models, we included the three-way interaction term 'species × sex × concentration' as well as the interaction terms 'species × concentration' and 'species × sex' to evaluate whether the species have different, concentration- or sex-dependent impacts. Furthermore, we selected the final models based on the Akaike Information Criterion (AIC). We performed linear models for the negative controls to analyze the baseline behavior, the overall dataset to see general trends between the species, and five individual models to account for intraspecies differences to the respective negative control. The statistical significance of explanatory variables in the linear models was evaluated by Type II ANOVAs (using the Anova function implemented in the car package (Fox et al., 2012)). Assumptions of normality of residuals were assessed visually by inspecting QQ plots and plots of residuals vs. fitted values. In addition, we performed Tukey's HSD post hoc tests to test for significant deviations between the negative controls and for each test concentration within the species. In this step, we calculated estimated marginal means (EMMs) to account for the influence of the explanatory variables. The graphical illustrations are based on these EMMs.

## 3. Results

### 3.1. Baseline activity

We found different baseline activities between the test species. The effects were differently expressed for the two different endpoints we used (TDM and TA; see Fig. 1). The total distance moved differed significantly between species ( $F_{152,4} = 4.13, p = 0.003$ ), but was not sex- ( $F_{152,1} = 2.24, p = 0.136$ ) or size- ( $F_{152,1} = 0.05, p = 0.817$ ) dependent (Table 1). Significantly different distances moved after the acclimatization phase were observed between uninfected *G. roeselii* and *G. fossarum* ( $p = 0.008$ ) and between uninfected and infected *G. roeselii*



**Fig. 1.** Baseline behavior of four amphipod species and infected *Gammarus roeselia* given as a) total distance moved per experiment and b) time spent active in the experiment. Values are based on estimated marginal means ( $\pm$ SE); the sample size is given in [Supplementary Table S1](#). Asterisks indicate significant deviations between the species, based on Tukey's HSD post hoc test (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). c) Combining total distance moved and time spent active. The trend line, forced to intersect the y-axis at  $y = 0$ , illustrates the linear association between the two variables and indicates a shift from fast movement to slow movement.

**Table 1**

Results of two separate general linear models on baseline behavior of four amphipod species using two different endpoints as dependent variables. Statistically significant effects are highlighted in bold.

Dependent variable	Independent variables	Df	F value	<i>p</i>
a) Total distance moved [m]	<b>species</b>	<b>4</b>	<b>4.13</b>	<b>0.003</b>
	sex	1	2.24	0.136
	size	1	0.05	0.817
b) Time active [%]	<b>species</b>	<b>4</b>	<b>12.43</b>	<b>&lt;0.001</b>
	sex	1	3.87	0.051
	size	1	0.07	0.788

( $p = 0.0446$ ). Uninfected *G. roeselia* swam the most, covering an estimated distance of 31.7 m during the 120 min test period, while the acanthocephalan-infected individuals of *G. roeselia* swam 17.8 m which is 56% of the maximal distance of uninfected *G. roeselia*. *D. villosus* and *G. pulex* did have similar TDM in baseline behavior with 26.0 m and 25.6 m, respectively. The native species *G. fossarum* showed with 12.4 m the least distance moved, which is 39% compared to the maximal distance of *G. roeselia*.

The time active differed significantly between the species ( $F_{152,4} = 12.5$ ,  $p < 0.001$ ), but not between sex ( $F_{152,1} = 3.87$ ,  $p = 0.051$ ) or size ( $F_{152,1} = 0.07$ ,  $p = 0.788$ ). *G. fossarum* was active 50.9% of the time, while *D. villosus* exhibited the lowest activity levels (active 35.3% of the time), demonstrating significantly ( $p < 0.001$ ) less active time compared to the other species (Fig. 1). Conversely, *G. pulex* was the most active species, being active 83.2% of the time. There were no significant differences in active time between non-infected and infected *G. roeselia* (71.7% and 75.4%, respectively).

If the two behavioral endpoints (TDM and TA) are plotted against each other, it becomes clear that there was reduced activity with fast movement in *D. villosus* (Fig. 1c). *G. roeselia* also showed a rather fast movement, but this shifted towards slow movement in animals infected with Acanthocephala.

### 3.2. Behavioral response to chemical exposure

The interaction term 'concentration  $\times$  species' had a significant effect on both, total distance moved and time active (TDM:  $F_{922,4} = 12.2$ ,  $p < 0.001$ ; TA:  $F_{922,4} = 66.5$ ,  $p < 0.001$ ; Table 2), indicating that the species showed different behavioral responses depending on the thiacloprid concentration. For both endpoints we found a significant impact

**Table 2**

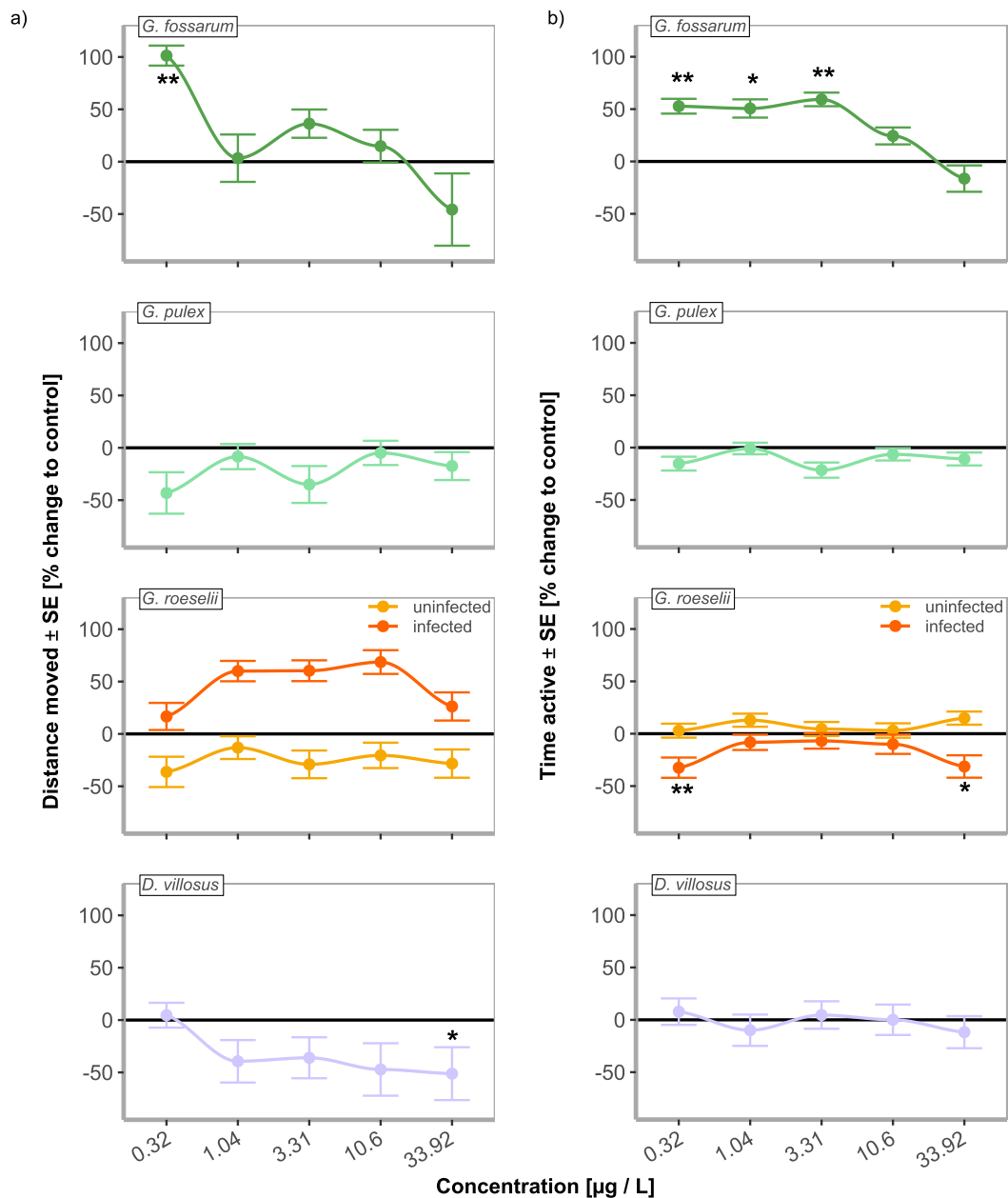
Results of two separate general linear models on behavioral patterns of four amphipod species using two different endpoints as dependent variables. Statistically significant effects are highlighted in bold.

Dependent variable	Independent variables	Df	F value	<i>p</i>
a) Total distance moved [m]	<b>species</b>	<b>4</b>	<b>12.24</b>	<b>&lt;0.001</b>
	<b>concentration</b>	<b>5</b>	<b>2.33</b>	<b>0.041</b>
	<b>sex</b>	<b>1</b>	<b>14.64</b>	<b>&lt;0.001</b>
	size	1	0.63	0.429
	<b>species <math>\times</math> concentration</b>	<b>20</b>	<b>3.16</b>	<b>&lt;0.001</b>
	<b>species <math>\times</math> sex</b>	<b>4</b>	<b>2.99</b>	<b>0.018</b>
b) Time active [%]	<b>species</b>	<b>4</b>	<b>66.47</b>	<b>&lt;0.001</b>
	concentration	5	2.21	0.051
	sex	1	0.07	0.796
	size	1	0.06	0.802
	<b>species <math>\times</math> concentration</b>	<b>20</b>	<b>3.23</b>	<b>&lt;0.001</b>
	<b>species <math>\times</math> sex</b>	<b>4</b>	<b>0.74</b>	<b>0.566</b>

of the interaction of the species with thiacloprid concentrations (TDM:  $F_{922,20} = 3.16$ ,  $p < 0.001$ ; TA:  $F_{922,20} = 3.23$ ,  $p < 0.001$ ) (Table 2). For TDM the interaction species  $\times$  sex had a significant impact ( $F_{922,4} = 2.99$ ,  $p = 0.018$ ; see [Supplementary Fig. S 1](#)).

Additional single-species linear models confirmed the significant effect of thiacloprid concentration on TDM for *G. fossarum* ( $F_{178,5} = 7.19$ ,  $p < 0.001$ ), *G. pulex* ( $F_{179,5} = 2.30$ ,  $p = 0.046$ ), and *D. villosus* ( $F_{178,5} = 3.77$ ,  $p = 0.003$ ; [Supplementary Table S 2](#)). *G. fossarum* showed a significant hyperactivity at the lowest concentration of thiacloprid (0.32  $\mu$ g/L; Fig. 2a), dropped back to the baseline activity at the medium concentrations and then showed a slight (statistically non-significant) hypoactivity at the highest concentration (33.92  $\mu$ g/L; for post hoc tests, see [Supplementary Table S 3](#)). We could not detect such hyperactivity for any other species. *D. villosus* showed a significantly reduced TDM (i.e., hypoactivity) at the highest concentration (Fig. 2a). In addition, the single-species models revealed a sex effect in *G. fossarum*, *G. pulex* and uninfected *G. roeselia* ([Supplementary Table S 2](#); [Supplementary Fig. S 2](#)).

The single-species models for the TA as second endpoint also confirmed significant effects of thiacloprid concentration ([Supplementary Table S 2](#)). Specifically, significant changes in activity were detected for *G. fossarum* ( $F_{178,5} = 8.23$ ,  $p < 0.001$ ), *G. pulex* ( $F_{179,5} = 2.59$ ,  $p = 0.027$ ) and the infected *G. roeselia* ( $F_{178,5} = 3.07$ ,  $p = 0.011$ ). The post hoc tests identified a significantly increased TA for *G. fossarum* at the



**Fig. 2.** Behavioral changes of four amphipod species upon exposure to the neonicotinoid thiacloprid. For *Gammarus roeselii*, individuals infected with *Acanthocephala* from the same sampling site were also considered. Shown are percentage deviations from the control, for the endpoints **a)** total distance moved (TDM) and **b)** relative time spent active [%] (TA). Values are based on estimated marginal means. Error bars show the standard error. Asterisks indicate significant deviations between the species, based on Tukey's HSD post hoc test (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; Table S 3).

first three concentrations (0.32–3.31  $\mu\text{g/L}$ ; for all post hoc comparisons, see [Supplementary Table S 3](#)). Infected *G. roeselii* showed a significantly reduced TA at the lowest (0.32  $\mu\text{g/L}$ ) and highest concentration of thiacloprid (33.92  $\mu\text{g/L}$ ). In contrast, uninfected *G. roeselii* showed no behavioral changes at the chosen thiacloprid concentrations (neither in endpoint TA nor TDM).

#### 4. Discussion

Our study highlights the behavioral diversity within closely related species, uncovers distinct baseline behaviors among amphipods, and emphasizes the nuanced, species-specific responses to thiacloprid exposure. This reveals a multifaceted web of behavioral intricacies, shedding light on the unique ways in which closely related species navigate and respond to environmental stressors.

##### 4.1. Species-specific baseline activity

Our expectation that baseline behavior differs between the investigated species was confirmed. *D. villosus*, described as a sit-and-wait predator (Maazouzi et al., 2011; Platvoet et al., 2009), was the least active species in terms of time active (TA). Interestingly, however, when active, they covered a large distance (reflected in the TDM, which did not differ from the other species). Contrary to our expectations, there were also clear differences between the other native and naturalized species, *G. fossarum* was significantly less active and covered less distance. The infected *G. roeselii* were moving less in a similar time, suggesting a slower movement behavior when active, possibly caused by the infection with acanthocephalans (see discussion below).

Studies that compare general behavioral differences between closely related amphipod species are rare. The most frequently addressed

species in behavioral studies is *D. villosus* and its comparison with native species, conforming that *D. villosus* moves more sporadically (Becker et al., 2016; Maazouzi et al., 2011; Platvoet et al., 2009). In contrast, Bierbach et al. (2016) analyzed the average swimming activity and found no difference between all the species considered in our study. So far, all studies vary in their protocol and our approach shows that the results depend on the behavioral endpoints considered. We conclude that the combination of active time and distance moved seems suitable to characterize the behavioral profile of the species. Possible differences in baseline behavior can be explained primarily by ecological differentiation. For example, in the study of Kohler et al. (2018) more specific behavioral endpoints (phototaxis, thigmotaxis and reaction to light stimuli) found differences between *G. pulex* and *Echinogammarus marinus*, attributed to their ecological differentiation. *E. marinus* depends on clinging to algae, a crucial food source, while *G. pulex* seeks organic material in the water flow (Kohler et al., 2018). The reduced activity of *G. fossarum* may be linked to its preference for upstream habitats, as elevated flow velocities in these areas likely prompt them to spend more time in sheltered locations to prevent passive drifting. To further clarify these species-specific behavioral profiles, additional endpoints such as phototaxis, microhabitat preferences and drift avoidance strategies would need to be characterized in future studies.

#### 4.2. Behavioral response to chemical exposure

We identified for the first time differently strong reactions to neonicotinoid exposure between closely related species with similar ecological backgrounds. Except for *G. pulex* and uninfected *G. roeselii*, all species showed some significant deviations from baseline activity. Thus, we could show that ecological similarity is not a predictor for the same response patterns.

We expected that pollution-naïve species react more pronounced to thiacloprid exposure compared to species that may be subject to chronic or recurrent exposure in their habitat. Indeed, *G. fossarum* exhibited a strong biphasic response pattern with hyperactivity (i.e., a flight response) at the lowest concentrations of thiacloprid that disappears at higher concentrations and then turns into hypoactivity. A biphasic response with high activity at low concentrations and low activity at high concentrations of the carbamate insecticide methomyl was also demonstrated by Xuereb et al. (2009) for *G. fossarum*. In their 96 h test with accompanying acetylcholinesterase (AChE) activity measurements, they found mode-of-action-related changes (i.e., 40% inhibition of locomotion activity and 66% inhibition of AChE compared to control) in behavioral response. Furthermore, Lebrun et al. (2020) demonstrated the behavioral sensitivity of *G. fossarum* towards the neonicotinoid imidacloprid, a substance with comparable toxicity and mode-of-action like thiacloprid (Morrissey et al., 2015). The study revealed stimulatory effects after 24 h, which the authors attributed to survival strategies in response to stress events, rather than mode-of-action related effects (Lebrun et al., 2020). The concentration employed by Lebrun et al. (2020) ranged between 0.1 and 1 µg/L, falling well within the spectrum covered by our first two concentrations (0.32 and 1.04 µg/L). Interestingly, all other species in our test reacted less markedly than *G. fossarum*. If the stimulating effects at low concentrations would be mode-of-action related, we would expect similar physiological responses in the four closely related species. As this is not the case, we also suspect stress as the reason for hyperactive behavior of *G. fossarum*. All species tended to show a slight decrease in activity with thiacloprid exposure, but this was not significant, except for *D. villosus* at the highest concentration (33.92 µg/L). *G. pulex* seems to be less reactive towards the tested neonicotinoid than to the previously tested carbamate pesticide methiocarb and the organophosphate dichlorvos (Soose et al., 2023). This shows that behavioral responses are specific to the test substance.

Hypoactive tendencies suggest a changed use of energy away from active avoidance towards maintaining vital functions such as ventilation or metabolic activities to passively avoid harm by the pollutant

(Gerhardt et al., 2005; Nørum et al., 2011; Peeters et al., 2009). Nevertheless, it remains possible that these subtle reductions in activity are indicative of early manifestation of mode-of-action-related effects which are expected to cause overstimulation of the nicotinic acetylcholine receptors and results in paralysis and if prolonged in death. Here further biomarker testing is needed to elucidate this hypothesis.

We cannot conclude whether this difference to *G. fossarum* is due to phylogenetic (i.e., species-specific) differences or pre-exposure and/or familiarity with the substance group. All species except *G. fossarum* occur in middle or lower sections of rivers that pass through agricultural regions and receive discharges from wastewater treatment plants – prior exposure to and thus familiarity with the substance group can therefore be expected (Beckers et al., 2020; Könemann et al., 2019; Švara et al., 2021). At the level of lethal endpoints, there is increasing evidence that recurrent or chronic exposure leads to adaptive changes and higher tolerance (Grethlein et al., 2022; Jourdan et al., 2024; Shahid et al., 2018; Siddique et al., 2020; Švara et al., 2021). This phenomenon is already observed at low, sublethal concentrations, as evidenced by a multigenerational study involving *Hyalella azteca* (Jourdan et al., 2024). However, such changes in tolerance have so far been described mainly for more tolerant species such as *G. roeselii* and *G. pulex*. *G. fossarum* did not show such patterns, but rather the opposite that pre-exposure lowers tolerance (i.e. leads to increased sensitivity; Grethlein et al., 2022; Zubrod et al., 2017). Assuming that a sudden change in behavior is potentially negative for the individual by causing active or passive drift and making individuals more prone to predation (Saenz et al., 2021; Sievers et al., 2018; Szokoli et al., 2015), a lack of sudden stress response could be beneficial and promote survival in contaminated waters. Adaptive behavioral responses, i.e. those that are not mode-of-action based and are not uncoordinated stress responses, can therefore be assumed for populations that have been exposed to pollutants before. Empirical evidence that links predation with individual behavioral changes is missing but would greatly improve our mechanistic understanding of the potentially increased predation risk during sudden behavioral changes.

#### 4.3. Acanthocephala infection impact

We expected to find increased activity in *G. roeselii* infected with acanthocephalans, but instead we found that activity remained the same, with individuals moving more slowly. Moreover, the response to the thiacloprid exposure varied in that infected *G. roeselii* tended to be hypoactive, but when active, they covered a large distance (i.e., showed fast movement). The reduced baseline activity observed in infected *G. roeselii* may stem from either passive effect, wherein the infection negatively impacted the gammarids health, or an active behavioral manipulation by the parasite. Behavior manipulation is specific for different host-parasite-systems and also depends on a co-evolutionary history (Moore, 1984). However, studies evidencing manipulation of *G. roeselii* by *P. laevis* are not to be found. Less co-evolutionary history between *P. laevis* and *G. roeselii* was used as explanation for the absence of behavioral manipulation by acanthocephalans in *G. roeselii* (Bauer et al., 2000). Behavior manipulation studies are often focused on phototactic, geotactic or clinging behavior instead of baseline behavior. For example, the altered phototactic behavior of *G. pulex* caused by infection with *P. laevis* has been well studied (Bauer et al., 2000; Tain et al., 2006). *P. minutus*, in contrast, influences clinging and geotactic behavior of *G. pulex* and *G. roeselii*, with a more pronounced impact observed on *G. pulex* (Bauer et al., 2005). Behavior of *G. roeselii* was furthermore impacted by *P. minutus* which improved non-host-predator-avoidance mechanisms and enhanced swimming speed (Medoc & Beisel, 2008). Our results now provide additional evidence that acanthocephalans also influence baseline behavior in *G. roeselii*. The reduced distance moved we observed contrasts with previous observations on *G. fossarum* and *P. minutus*, where an increase in mean overall activity (measured in Hz) was observed (Rothe et al.,

2022). If an increased activity – as observed for *G. fossarum* – was caused by an active manipulation of the intermediate host, we argue that the reduction we observed can rather be explained by an indirect effect of the acanthocephalan infection. Such indirect effect would not be caused by active manipulation, but by metabolic changes in the intermediate host *G. roeselii*. For example, Rothe et al. (2022) and Gismondi et al. (2012) found increased activity of antitoxic biomarkers in gammarids infected with *P. minutus*, indicating possible impact of acanthocephalans on subcellular mechanisms. In which direction this might alter the behavior of *G. roeselii* cannot be explained conclusively as it must be noted that our current understanding of the impacts of acanthocephalans on *Gammarus* spp. is predominantly centered around *P. minutus*, with a notable gap in knowledge regarding the effects of *P. laevis* and its intermediate hosts such as *G. roeselii*.

A decrease in baseline behavior could also account for the high prevalence of *P. laevis*, reaching up to 73% in *G. roeselii* within the study area (Kochmann et al., 2023). Because – in contrast to *G. fossarum* – the slower movement may reduce the risk of predation, it favors the survival of infected *G. roeselii* and thus contributes to the high prevalence. Moreover, like the observed increased tolerance of infected *G. roeselii* to acute exposure to the pyrethroid deltamethrin (Kochmann et al., 2023), we also observed an altered behavioral response to thiacloprid exposure. Infected *G. roeselii* reduced activity and tended to move faster – an effect we did not observe in uninfected individuals. We do not yet have an explanation for the observations, but it is becoming increasingly evident that acanthocephalan infection affects various physiological levels in the amphipod intermediate host, which significantly shapes their survival in polluted habitats. Investigating the intricate interplay of stress factors, including acanthocephalan infection and chemical pollution, not only underscores the complexity of ecological interactions under chemical stress but also represents a crucial focus for future research to unravel the nuanced dynamics of host-parasite relationships and their broader ecological impacts.

#### 4.4. Environmental relevance

The question of ecological relevance is one that behavioral ecotoxicology studies are often confronted with. At the same time, we are observing massive biodiversity losses, which are primarily expressed in a restructuring of species communities – more than in changing diversity metrics (Enns et al., 2023). Interestingly, our findings align with monitoring results, revealing that *G. fossarum*, a species considered to be very sensitive to chemical pollution (Adam et al., 2010; Enns et al., 2023), exhibits the most pronounced behavioral changes in our assessment. Our experiments revealed these behavioral changes already within measured environmental concentration ranges between 0.7 and 4.69 µg/L (Betz-Koch et al., 2023; IPChem Portal, 2022). For now, we lack a mechanistic explanation for how the change in behavior negatively affects survival. Yet, it seems obvious that different behavioral responses also contribute to how a population survives under chemical stress and can even numerically dominate the invertebrate community in polluted areas (Enns et al., 2023; Peterson et al., 2017). In theory, we assume that untypical behavior after chemical exposure, independent of whether it is mode-of-action related or stress-based, can lead to increased predation risk or drift events. Evidence comes from Beketov and Liess (2008), who showed that short-term pulses of thiacloprid among other neonicotinoids initiated drift events in sensitive taxa. Drift was initiated at 30.3 µg/L which is close to our highest test concentration (33.92 µg/L). In a comparative study by Nørnum et al. (2010), both laboratory locomotion and mesocosm drift experiments, provided evidence of chemically induced behavioral changes in different invertebrates causing drift events. The study suggests that hyperactivity as well as hypoactivity contribute to drift.

Invasive species are often considered to be a major driver of biodiversity change (Roy et al., 2023), but the question also arises as to what extent they may be the consequence of altered ecosystems in which

native species are already significantly weakened (J.T. Bauer, 2012; Didham et al., 2005). To answer this question, it is essential to test the persistence of invasive species and their natural counterparts, which have been displaced in many regions, in the face of environmental pressures. Once successful, this will not only improve our understanding of species turnover but also allow invasive species to be considered as bioindicators. The omnipresent phenomenon of invasive species can therefore be a prime example of the repeatedly demanded need to link ecological observations more closely with ecotoxicological approaches (Gessner & Tlili, 2016; Sylvester et al., 2023). The example of *D. villosus* shows that they differ from their replaced counterparts by a higher fecundity and omnivory (Platvoet et al., 2009; Pöckl, 2009). Elucidating whether its success stems solely from these attributes or also involves variances in pollutant tolerance could offer additional insights into the mechanisms driving invasion success of *D. villosus*. Our results confirm on the one hand that *D. villosus* has a different baseline behavior compared to the native species and on the other hand they suggest that *D. villosus* shows a different behavioral response to the chemicals at least compared to *G. fossarum*. We are thus addressing a knowledge gap that is still large, and the example of *D. villosus* shows that it is worth investigating the response to chemical stress, especially in comparison to potentially threatened species (Boets et al., 2012; Sornom et al., 2012). Rare comparative studies showed contrasting results, with *D. villosus* being less tolerant to cadmium (Boets et al., 2012) and more tolerant to copper and the insecticide lambda-cyhalothrin than other species (Bundschuh et al., 2013; Sroda & Cossu-Leguille, 2011). We are only at the beginning and future studies ought to paint a more complete picture of the susceptibility of ecologically significant invasive and native species found locally. This will help to understand ecological phenomena of species turnover and also to utilize the potential of invasive species as bioindicators.

## 5. Conclusion

We presented a promising and ecologically realistic behavioral monitoring approach that was sensitive to assess the species-specific behavioral response of gammarids under sublethal exposure to neonicotinoid contamination. The results underscore that ecologically similar and closely related species respond differently to pesticide exposure, hindering inferences from one species to another. In addition, species interactions, such as parasite-host dynamics, continue to influence behavioral responses. This study marks the next steps in routine behavioral monitoring, demonstrating the potential of behavioral studies and providing insight into sublethal responses of invertebrate key species. Species-specific behavioral responses could contribute to the explanation why we observe a turnover of ecologically similar and closely related species in many anthropogenic shaped environments.

### CRedit authorship contribution statement

**Laura J. Soose:** Investigation, Conceptualization, Formal analysis, Data Curation, Methodology, Visualization, Writing – original draft, Writing – review and editing; **Tobias Rex:** Investigation, Formal analysis, Data Curation, Writing – review and editing; **Jörg Oehlmann:** Resources, Methodology, Writing – review and editing; **Andreas Schiwiy:** Methodology, Writing – review and editing; **Martin Krauss:** Methodology, Writing – review and editing; **Werner Brack:** Writing – review and editing; **Sven Klimpel:** Resources, Writing – review and editing; **Henner Hollert:** Resources, Writing – review and editing; **Jonas Jourdan:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review and editing.

### 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.

## Data availability

The data that supports the findings of this study is available via figshare (Soose et al., 2024).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.124235>.

## References

- Adam, O., Degiorgi, F., Crini, G., Badot, P.-M., 2010. High sensitivity of *Gammarus* sp. Juveniles to deltamethrin: outcomes for risk assessment. *Ecotoxicol. Environ. Saf.* 73 (6), 1402–1407. <https://doi.org/10.1016/j.ecoenv.2010.02.011>.
- Ågerstrand, M., Arnold, K., Balshine, S., Brodin, T., Brooks, B.W., Maack, G., McCallum, E.S., Pyle, G., Saaristo, M., Ford, A.T., 2020. Emerging investigator series: use of behavioural endpoints in the regulation of chemicals. *Environmental Science. Processes & Impacts* 22 (1), 49–65. <https://doi.org/10.1039/c9em00463g>.
- Albert, J.S., Destouni, G., Duke-Sylvester, S.M., Magurran, A.E., Oberdorff, T., Reis, R.E., Winemiller, K.O., Ripple, W.J., 2021. Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio* 50 (1), 85–94. <https://doi.org/10.1007/s13280-020-01318-8>.
- Amiard-Triquet, C., 2015. Chapter 6 - how to improve toxicity assessment? From single-species tests to mesocosms and field studies. In: Amiard-Triquet, C., Amiard, J.-C., Mouneyrac, C. (Eds.), *Aquatic Ecotoxicology*. Academic Press, pp. 127–151. <https://doi.org/10.1016/B978-0-12-800949-9.00006-1>.
- Baranov, V., Jourdan, J., Pilotto, F., Wagner, R., Haase, P., 2020. Complex and nonlinear climate-driven changes in freshwater insect communities over 42 years. *Conserv. Biol.* 34 (5), 1241–1251. <https://doi.org/10.1111/cobi.13477>.
- Bauer, A., Haine, E.R., Perrot-Minnot, M., Rigaud, T., 2005. The acanthocephalan parasite *Polymorphus minutus* alters the geotactic and clinging behaviours of two sympatric amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeselii*. *J. Zool.* 267 (1), 39–43. <https://doi.org/10.1017/S0952836905007223>.
- Bauer, A., Trouvé, S., Grégoire, A., Bollache, L., Cézilly, F., 2000. Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. *Int. J. Parasitol.* 30 (14), 1453–1457. [https://doi.org/10.1016/S0020-7519\(00\)00138-7](https://doi.org/10.1016/S0020-7519(00)00138-7).
- Bauer, J.T., 2012. Invasive species: “Back-seat drivers” of ecosystem change? *Biol. Invasions* 14 (7), 1295–1304. <https://doi.org/10.1007/s10530-011-0165-x>.
- Becker, J., Ortmann, C., Wetzel, M.A., Koop, J.H.E., 2016. Metabolic activity and behavior of the invasive amphipod *Dikerogammarus villosus* and two common Central European gammarid species (*Gammarus fossarum*, *Gammarus roeselii*): low metabolic rates may favor the invader. *Comp. Biochem. Physiol. Mol. Integr. Physiol.* 191, 119–126. <https://doi.org/10.1016/j.cbpa.2015.10.015>.
- Beckers, L.-M., Brack, W., Dann, J.P., Krauss, M., Müller, E., Schulze, T., 2020. Unraveling longitudinal pollution patterns of organic micropollutants in a river by non-target screening and cluster analysis. *Sci. Total Environ.* 727, 138388. <https://doi.org/10.1016/j.scitotenv.2020.138388>.
- Beketov, M.A., Liess, M., 2008. Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. *Arch. Environ. Contam. Toxicol.* 55 (2), 247–253. <https://doi.org/10.1007/s00244-007-9104-3>.
- Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. *Front. Ecol. Environ.* 15 (2), 84–90. <https://doi.org/10.1002/fee.1450>.
- Bertram, M.G., Martin, J.M., McCallum, E.S., Alton, L.A., Brand, J.A., Brooks, B.W., Cervený, D., Fick, J., Ford, A.T., Hellström, G., Michelangeli, M., Nakagawa, S., Polverino, G., Saaristo, M., Sih, A., Tan, H., Tyler, C.R., Wong, B.B.M., Brodin, T., 2022. Frontiers in quantifying wildlife behavioural responses to chemical pollution. *Biol. Rev.* 97 (4), 1346–1364. <https://doi.org/10.1111/brv.12844>.
- Betz-Koch, S., Jacobs, B., Oehlmann, J., Ratz, D., Reutter, C., Wick, A., Oetken, M., 2023. Pesticide dynamics in three small agricultural creeks in Hesse, Germany. *PeerJ* 11, e15650. <https://doi.org/10.7717/peerj.15650>.
- Bierbach, D., Laskowski, K.L., Brandt, A.-L., Chen, W., Jourdan, J., Streit, B., Plath, M., 2016. Highly variable, unpredictable activity patterns in invasive, but not native amphipod species. *Aquat. Ecol.* 50 (2), 261–271. <https://doi.org/10.1007/s10452-016-9573-4>.
- Bij de Vaate, A., Jazdzewski, K., Ketelaars, H.A.M., Gollasch, S., Van der Velde, G., 2002. Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Can. J. Fish. Aquat. Sci.* 59 (7), 1159–1174. <https://doi.org/10.1139/f02-098>.
- Boets, P., Lock, K., Goethals, P.L.M., Janssen, C.R., De Schampelaere, K.A.C., 2012. A comparison of the short-term toxicity of cadmium to indigenous and alien gammarid species. *Ecotoxicology* 21 (4), 1135–1144. <https://doi.org/10.1007/s10646-012-0868-5>.
- Brack, W., Aissa, S.A., Backhaus, T., Dulio, V., Escher, B.I., Faust, M., Hilscherova, K., Hollender, J., Hollert, H., Müller, C., Munthe, J., Posthuma, L., Seiler, T.-B., Slobodnik, J., Teodorovic, I., Tindall, A.J., de Aragão Umbuzeiro, G., Zhang, X., Altenburger, R., 2019. Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. *Environ. Sci. Eur.* 31 (1), 10. <https://doi.org/10.1186/s12302-019-0192-2>.
- Breitholtz, M., Rudén, C., Ove Hansson, S., Bengtsson, B.-E., 2006. Ten challenges for improved ecotoxicological testing in environmental risk assessment. *Ecotoxicol. Environ. Saf.* 63 (2), 324–335. <https://doi.org/10.1016/j.ecoenv.2005.12.009>.
- Brinkmann, M., Montgomery, D., Selinger, S., Miller, J.G.P., Stock, E., Alcaraz, A.J., Challis, J.K., Weber, L., Janz, D., Hecker, M., Wiseman, S., 2022. Acute toxicity of the tire rubber-derived chemical 6PPD-quinone to four fishes of commercial, cultural, and ecological importance. *Environ. Sci. Technol. Lett.* 9 (4), 333–338. <https://doi.org/10.1021/acs.estlett.2c00050>.
- Bundschuh, M., Gergs, R., Schadt, S., Schulz, R., 2013. Do differences in sensitivity between native and invasive amphipods explain their coexistence in Lake Constance? A case study with lambda-cyhalothrin. *Chemosphere* 92 (5), 483–489. <https://doi.org/10.1016/j.chemosphere.2013.01.106>.
- Bundschuh, M., Zubrod, J.P., Klöttschen, S., Englert, D., Schulz, R., 2020. Infochemicals influence neonicotinoid toxicity-impact in leaf consumption, growth, and predation of the amphipod *Gammarus fossarum*. *Environ. Toxicol. Chem.* 39 (9), 1755–1764. <https://doi.org/10.1002/etc.4802>.
- Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W., Almond, R.E.A., Baillie, J.E.M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K.E., Carr, G.M., Chanson, J., Chenery, A.M., Csirke, J., Davidson, N.C., Dentener, F., Foster, M., Galli, A., et al., 2010. Global biodiversity: indicators of recent declines. *Science* 328 (5982), 1164–1168. <https://doi.org/10.1126/science.1187512>.
- Candolin, U., Wong, B.B.M., 2019. Mate choice in a polluted world: consequences for individuals, populations and communities. *Phil. Trans. Roy. Soc. Lond. B Biol. Sci.* 374 (1781), 20180055. <https://doi.org/10.1098/rstb.2018.0055>.
- Chen, K., Liu, X., Wu, X., Xu, J., Dong, F., Zheng, Y., 2021. The degradation dynamics and rapid detection of thiacloprid and its degradation products in water and soil by UHPLC-QTOF-MS. *Chemosphere* 263, 127960. <https://doi.org/10.1016/j.chemosphere.2020.127960>.
- Copilaş-Ciocianu, D., Borko, Ş., Fiser, C., 2020. The late blooming amphipods: global change promoted post-Jurassic ecological radiation despite Palaeozoic origin. *Mol. Phylogenet. Evol.* 143, 106664. <https://doi.org/10.1016/j.ympev.2019.106664>.
- Csapó, H., Krzywoźniak, P., Grabowski, M., Wattier, R., Bącela-Spychalska, K., Mamos, T., Jelić, M., Rewicz, T., 2020. Successful post-glacial colonization of Europe by single lineage of freshwater amphipod from its Pannonian Plio-Pleistocene diversification hotspot. *Sci. Rep.* 10 (1) <https://doi.org/10.1038/s41598-020-75568-7>. Article 1.
- Cui, R., Kwak, J.I., An, Y.-J., 2018. Comparative study of the sensitivity of *Daphnia galeata* and *Daphnia magna* to heavy metals. *Ecotoxicol. Environ. Saf.* 162, 63–70. <https://doi.org/10.1016/j.ecoenv.2018.06.054>.
- Dalla Bona, M., Di Leva, V., De Liguoro, M., 2014. The sensitivity of *Daphnia magna* and *Daphnia curvirostris* to 10 veterinary antibacterials and to some of their binary mixtures. *Chemosphere* 115, 67–74. <https://doi.org/10.1016/j.chemosphere.2014.02.003>.
- De Lange, H.J., Noordoven, W., Murk, A.J., Lürling, M., Peeters, E.T.H.M., 2006. Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. *Aquat. Toxicol.* 78 (3), 209–216. <https://doi.org/10.1016/j.aquatox.2006.03.002>.
- Devin, S., Piscart, C., Beisel, J.N., Moreteau, J.C., 2003. Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. *Arch. Hydrobiol.* 158 (1), 43–56. <https://doi.org/10.1127/0003-9136/2003/0158-0043>.
- Didham, R.K., Tylianakis, J.M., Hutchison, M.A., Ewers, R.M., Gemmill, N.J., 2005. Are invasive species the drivers of ecological change? *Trends Ecol. Evol.* 20 (9), 470–474. <https://doi.org/10.1016/j.tree.2005.07.006>.
- EFSA Panel on Plant Protection Products and their Residues (PPR), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA J.* 11 (7) <https://doi.org/10.2903/j.efsa.2013.3290>.
- Enns, D., Cunze, S., Baker, N.J., Oehlmann, J., Jourdan, J., 2023. Flushing away the future: the effects of wastewater treatment plants on aquatic invertebrates. *Water Res.* 243, 120388. <https://doi.org/10.1016/j.watres.2023.120388>.
- European Commission, 2008. Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC. Assessment report thiacloprid. [http://dissemination.echa.europa.eu/Biocides/ActiveSubstances/0053-08/0053-08\\_Assessment\\_Report.pdf](http://dissemination.echa.europa.eu/Biocides/ActiveSubstances/0053-08/0053-08_Assessment_Report.pdf).
- European Union, 2023. EU pesticides database—active substances. <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances>.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3 (5), 294–299.
- Ford, A.T., Ågerstrand, M., Brooks, B.W., Allen, J., Bertram, M.G., Brodin, T., Dang, Z., Duquesne, S., Sahn, R., Hoffmann, F., Hollert, H., Jacob, S., Klüver, N., Lazorchak, J. M., Ledesma, M., Melvin, S.D., Mohr, S., Padilla, S., Pyle, G.G., et al., 2021. The role of behavioral ecotoxicology in environmental protection. *Environ. Sci. Technol.* <https://doi.org/10.1021/acs.est.0c06493>.



- Pelikan, L., Šidagytė-Copilas, E., Garbaras, A., Jourdan, J., Copilaș-Ciocianu, D., 2024. Competitive interaction in headwaters: slow upstream migration leads to trophic competition between native and non-native amphipods. *Neobiota* 90, 193–216. <https://doi.org/10.3897/neobiota.90.112383>.
- Peterson, E.K., Buchwalter, D.B., Kerby, J.L., LeFauve, M.K., Varian-Ramos, C.W., Swaddle, J.P., 2017. Integrative behavioral ecotoxicology: bringing together fields to establish new insight to behavioral ecology, toxicology, and conservation. *Current Zoology* 63 (2), 185–194. <https://doi.org/10.1093/cz/zox010>.
- Piscart, C., Roussel, J.-M., Dick, J.T.A., Grosbois, G., Marmonier, P., 2011. Effects of coexistence on habitat use and trophic ecology of interacting native and invasive amphipods. *Freshw. Biol.* 56 (2), 325–334. <https://doi.org/10.1111/j.1365-2427.2010.02500.x>.
- Platvoet, D., Van Der Velde, G., Dick, J.T.A., Li, S., 2009. Flexible omnivory in *Dikerogammarus villosus* (Sowinsky, 1894) (Amphipoda)—amphipod pilot species project (AMPIS) report 5. *Crustaceana* 82 (6), 703–720. <https://www.jstor.org/stable/27743326>.
- Pöckl, M., 1993. Reproductive potential and lifetime potential fecundity of the freshwater amphipods *Gammarus fossarum* and *G. roeseli* in Austrian streams and rivers. *Freshw. Biol.* 30 (1), 73–91. <https://doi.org/10.1111/j.1365-2427.1993.tb00790.x>.
- Pöckl, M., 2009. Success of the invasive Ponto-Caspian amphipod *Dikerogammarus villosus* by life history traits and reproductive capacity. *Biol. Invasions* 11 (9), 2021–2041. <https://doi.org/10.1007/s10530-009-9485-5>.
- Podwysocki, K., Baćela-Spychalska, K., Desiderato, A., Rewicz, T., Copilaș-Ciocianu, D., 2024. Environment, intraspecific lineages and geographic range jointly shape the high morphological variability of *Dikerogammarus villosus* (Sowinsky, 1894) (Crustacea, Amphipoda): a successful aquatic invader across Europe. *Hydrobiologia*. <https://doi.org/10.1007/s10750-024-05565-8>.
- Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., McCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol. Rev.* 94 (3), 849–873. <https://doi.org/10.1111/brv.12480>.
- Rewicz, T., Grabowski, M., MacNeil, C., Baćela-Spychalska, K., 2014. The profile of a 'perfect' invader – the case of killer shrimp, *Dikerogammarus villosus*. *Aquat. Invasions* 9 (3), 267–288. <https://doi.org/10.3391/ai.2014.9.3.04>.
- Rewicz, T., Wattier, R., Grabowski, M., Rigaud, T., Baćela-Spychalska, K., 2015. Out of the black sea: phylogeography of the invasive killer shrimp *Dikerogammarus villosus* across Europe. *PLoS One* 10 (2), e0118121. <https://doi.org/10.1371/journal.pone.0118121>.
- Rothe, L.E., Loeffler, F., Gerhardt, A., Feld, C.K., Stift, R., Weyand, M., Grabner, D., Sures, B., 2022. Parasite infection influences the biomarker response and locomotor activity of *Gammarus fossarum* exposed to conventionally-treated wastewater. *Ecotoxicol. Environ. Saf.* 236, 113474. <https://doi.org/10.1016/j.ecoenv.2022.113474>.
- Roy, H.E., Pauchard, A., Stoett, P., Renard Truong, T., Bacher, S., Galil, B.S., Hulme, P.E., Ikeda, T., Sankaran, K.V., McGeoch, M.A., Meyerson, L.A., Nuñez, M.A., Ordóñez, A., Rahlaoui, S.J., Schwindt, E., Seebens, H., Sheppard, A.W., Vandvik, V., *IPBES invasive alien species assessment: Summary for policymakers*. Zenodo. <https://doi.org/10.5281/zenodo.8314303>.
- Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M., Arnold, K.E., 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proc. R. Soc. Ser. B: Biol. Sci.* 285 (1885), 20181297. <https://doi.org/10.1098/rspb.2018.1297>.
- Saenz, D., Baum, K.A., Fitzgerald, L.A., Schalk, C.M., 2021. Refining the activity level-predation risk paradigm in larval anuran communities. *Aquat. Sci.* 83 (1), 4. <https://doi.org/10.1007/s00027-020-00752-w>.
- Santos-Medrano, G.E., Rico-Martinez, R., 2019. Acute sensitivity comparison among *Daphnia magna* Straus, 1820 *Daphnia pulex* Leydig, 1860 and *Simocephalus vetulus* Müller, 1776, exposed to nine toxicants. *Turk. J. Fish. Aquat. Sci.* 19 (7), 615–623. [https://doi.org/10.4194/1303-2712-v19\\_7\\_08](https://doi.org/10.4194/1303-2712-v19_7_08).
- Schmidt-Rhaesa, 2015. *Gastrotricha, Cycloneuralia and Gnathifera. Gastrotricha and Gnathifera*, 3. Walter de Gruyter GmbH (Handbook of Zoology, Berlin, Germany, Munich, Germany, Boston, Massachusetts).
- Shahid, N., Becker, J.M., Krauss, M., Brack, W., Liess, M., 2018. Adaptation of *Gammarus pulex* to agricultural insecticide contamination in streams. *Sci. Total Environ.* 621, 479–485. <https://doi.org/10.1016/j.scitotenv.2017.11.220>.
- Siddique, A., Liess, M., Shahid, N., Becker, J.M., 2020. Insecticides in agricultural streams exert pressure for adaptation but impair performance in *Gammarus pulex* at regulatory acceptable concentrations. *Sci. Total Environ.* 722, 137750. <https://doi.org/10.1016/j.scitotenv.2020.137750>.
- Sievers, M., Hale, R., Swearer, S.E., Parris, K.M., 2018. Contaminant mixtures interact to impair predator-avoidance behaviours and survival in a larval amphibian. *Ecotoxicol. Environ. Saf.* 161, 482–488. <https://doi.org/10.1016/j.ecoenv.2018.06.028>.
- Sigma Aldrich, 2023. Safety data sheet according to regulation (EC) No. 1907/2006, Version 6.6. <https://www.sigmaaldrich.com/DE/en/sds/sial/37905?userType=anonymous>.
- Sigmund, G., Ågerstrand, M., Antonelli, A., Backhaus, T., Brodin, T., Diamond, M.L., Erdelen, W.R., Evers, D.C., Hofmann, T., Hueffer, T., Lai, A., Torres, J.P.M., Mueller, L., Perrigo, A.L., Rillig, M.C., Schaeffer, A., Scheringer, M., Schirmer, K., Tlili, A., et al., 2023. Addressing chemical pollution in biodiversity research. *Global Change Biol.* 29 (12), 3240–3255. <https://doi.org/10.1111/gcb.16689>.
- Soose, L.J., Hügl, K.S., Oehlmann, J., Schiwuy, A., Hollert, H., Jourdan, J., 2023. A novel approach for the assessment of invertebrate behavior and its use in behavioral ecotoxicology. *Sci. Total Environ.* 897, 165418. <https://doi.org/10.1016/j.scitotenv.2023.165418>.
- [dataset] Soose, L. J., Rex, T., Oehlmann, J., Schiwuy, A., Krauss, M., Brack, W., Klimpel, S., Hollert, H., Jourdan, J., 2024. *Dataset: One like all? Behavioral response range of native and invasive amphipods to neonicotinoid exposure* (Version 1). figshare. <https://doi.org/10.6084/m9.figshare.25295608>.
- Sornom, P., Gismondi, E., Vellinger, C., Devin, S., Féraud, J.-F., Beisel, J.-N., 2012. Effects of sublethal cadmium exposure on antipredator behavioural and antitoxic responses in the invasive amphipod *Dikerogammarus villosus*. *PLoS One* 7 (8), e42435. <https://doi.org/10.1371/journal.pone.0042435>.
- Soto, I., Cuthbert, R.N., Ricciardi, A., Ahmed, D.A., Altermatt, F., Schäfer, R.B., Archambaud-Suard, G., Bonada, N., Cañedo-Argüelles, M., Csabai, Z., Datry, T., Dick, J.T.A., Floury, M., Forio, M.A.E., Forcellini, M., Frugot, J.-F., Goethals, P., Haase, P., Hudgins, E.J., et al., 2023. The faunal Ponto-Caspianization of central and western European waterways. *Biol. Invasions* 25 (8), 2613–2629. <https://doi.org/10.1007/s10530-023-03060-0>.
- Sroda, S., Cossu-Leguille, C., 2011. Effects of sublethal copper exposure on two gammarid species: which is the best competitor? *Ecotoxicology* 20 (1), 264–273. <https://doi.org/10.1007/s10646-010-0578-9>.
- Švara, V., Krauss, M., Michalski, S.G., Altenburger, R., Brack, W., Luckenbach, T., 2021. Chemical pollution levels in a river explain site-specific sensitivities to micropollutants within a genetically homogeneous population of freshwater amphipods. *Environ. Sci. Technol.* 55 (9), 6087–6096. <https://doi.org/10.1021/acs.est.0c07839>.
- Sylvester, F., Weichert, F.G., Lozano, V.L., Groh, K.J., Bálint, M., Baumann, L., Bässler, C., Brack, W., Brandl, B., Curtius, J., Dierkes, P., Döll, P., Ebersberger, I., Fragkostefanakis, S., Helfrich, E.J.N., Hickler, T., Johann, S., Jourdan, J., Klimpel, S., et al., 2023. Better integration of chemical pollution research will further our understanding of biodiversity loss. *Nature Ecology & Evolution* 7, 1552–1555. <https://doi.org/10.1038/s41559-023-02117-6>.
- Szokoli, F., Winkelmann, C., Berendonk, T.U., Worischka, S., 2015. The effects of fish kairomones and food availability on the predator avoidance behaviour of *Gammarus pulex*. *Fundamental and Applied Limnology* 186 (3), 249–258. <https://doi.org/10.1127/fal/2015/0633>.
- Tain, L., Perrot-Minnot, M.-J., Cézilly, F., 2006. Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proc. Biol. Sci.* 273 (1605), 3039–3045. <https://doi.org/10.1098/rspb.2006.3618>.
- Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., et al., 2021. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science* 371 (6525), 185–189. <https://doi.org/10.1126/science.abd6951>.
- Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu. Rev. Pharmacol. Toxicol.* 45, 247–268. <https://doi.org/10.1146/annurev.pharmtox.45.120403.095930>.
- U.S. EPA, 2003. Fact Sheet for Thiachloprid. [https://www3.epa.gov/pesticides/chem\\_srch/reg\\_actions/registration/fs\\_PC-014019\\_26-Sep-03.pdf](https://www3.epa.gov/pesticides/chem_srch/reg_actions/registration/fs_PC-014019_26-Sep-03.pdf).
- Van Riel, M.C., Van der Velde, G., Bij de Vaate, A., 2011. Dispersal of invasive species by drifting. *Current Zoology - CURR ZOOL* 57, 818–827. <https://doi.org/10.1093/czoolo/57.6.818>.
- Vighi, M., Villa, S., 2013. Ecotoxicology: the challenges for the 21st century. *Toxics* 1 (1), 18–35. <https://doi.org/10.3390/toxics1010018>.
- Von der Ohe, P.C., Liess, M., 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. *Environ. Toxicol. Chem.* 23 (1), 150–156. <https://doi.org/10.1897/02-577>.
- Wattier, R., Mamos, T., Copilaș-Ciocianu, D., Jelić, M., Ollivier, A., Chaumot, A., Danger, M., Felten, V., Piscart, C., Žganeč, K., Rewicz, T., Wysocka, A., Rigaud, T., Grabowski, M., 2020. Continental-scale patterns of hyper-cryptic diversity within the freshwater model taxon *Gammarus fossarum* (Crustacea, Amphipoda). *Sci. Rep.* 10 (1), 16536. <https://doi.org/10.1038/s41598-020-73739-0>.
- Weigand, A.M., Michler-Kozma, D., Kuemmerlen, M., Jourdan, J., 2020. Substantial differences in genetic diversity and spatial structuring among (cryptic) amphipod species in a mountainous river basin. *Freshw. Biol.* 65 (9), 1641–1656. <https://doi.org/10.1111/fwb.13529>.
- Xuereb, B., Lefevre, E., Garric, J., Geffard, O., 2009. Acetylcholinesterase activity in *Gammarus fossarum* (Crustacea Amphipoda): linking AChE inhibition and behavioural alteration. *Aquat. Toxicol.* 94 (2), 114–122. <https://doi.org/10.1016/j.aquatox.2009.06.010>.
- Zubrod, J.P., Englert, D., Lüderwald, S., Poganiuch, S., Schulz, R., Bundschuh, M., 2017. History matters: Pre-exposure to wastewater enhances pesticide toxicity in invertebrates. *Environ. Sci. Technol.* 51 (16), 9280–9287. <https://doi.org/10.1021/acs.est.7b01303>.