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Infochemicals Influence Neonicotinoid Toxicity—Impact in Leaf Consumption, Growth, and Predation of the Amphipod *Gammarus fossarum*

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Abstract: Infochemicals act as inter- or intraspecific messengers. The literature suggests complex interactions between infochemicals (mainly predator cues) and chemical (e.g., pesticide) effects, with their direction and magnitude depending on the cue origin, pesticide identity, and test species. With the present study we assessed the impact of alarm cues alone and in combination with the neonicotinoid insecticide thiacloprid on leaf consumption, predation on *Baetis* nymphs, and dry weight of the amphipod *Gammarus fossarum*. Alarm cues (ground gammarids) and thiacloprid alone decreased gammarid leaf consumption with increasing intensities. At a defined alarm cue intensity, which alone did not cause a significant reduction in gammarid feeding, thiacloprid-induced feeding effects were additive. During an experiment targeting gammarid predation on *Baetis* nymphs (120 h), thiacloprid and alarm cues alone did increase and reduce predation significantly, respectively. Moreover, alarm cues led to a lower final gammarid dry weight. However, alarm cues did not affect response variables during a second predation experiment performed at a higher thiacloprid concentration (2 vs 0.75 µg/L). This discrepancy in alarm cue effects highlights either a varying susceptibility of the test species to these cues among experiments or that cue quality is fluctuating. Thus, the present study highlights a considerable variability in the individual and interactive effects of infochemicals and chemical stressors on aquatic biota, an insight relevant in the assessment of multiple stressors. *Environ Toxicol Chem* 2020;39:1755–1764. © 2020 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Environmental stimulus; Odor; Predator–prey interaction; Conspecific alarm cue; Semiochemical

INTRODUCTION

Organisms communicate, among other ways, by the release of chemical messengers, so-called infochemicals. These conspecific chemicals or cues help individuals of the same species to find food, to mate, to mark territories, or to warn of danger (Dicke and Takken 2006). Moreover, heterospecific cues allow for communication between species, for instance, with predatory cues (i.e., kairomones) informing about the presence of predators, leading to defensive responses in prey species (Barry 1998). A well-known phenomenon in freshwater ecosystems is the formation of a crown or helmet by *Daphnia* in response to predatory cues (Hanazato 1991; Petrusek et al. 2009). Chemical stressors of anthropogenic origin can, however, disrupt the transfer of information through infochemicals (i.e., act as "info-disruptors"). Besides heavy metals, pesticides and other classes of organic chemicals, such as surfactants, have the potential to act as such info-disruptors (Lurling and Scheffer 2007). Indeed, the pyrethroid insecticide cyfluthrin affected a parasitoid's ability (i.e., *Telenomus busseolae*) to respond to host cues, whereas another pyrethroid insecticide, deltamethrin, did not cause comparable effects (Bayram et al. 2010). In contrast, deltamethrin inhibited the binding of pheromones to respective receptors in *Trichogramma semblidis*, another parasitoid. At the same time, this insecticide was ineffective in the related species *Trichogramma evanescens* (Delpuech et al. 2012). Endosulfan, an organochlorine insecticide, positively influenced crown development (and thus antipredator behavior) in *Daphnia* when combined with predator cues of the backswimmer *Anisops gratus* (Barry 1998). The opposite, namely an impaired phenotypic response of *Bufo arabicus* tadpoles to predator cues, was

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observed when exposed to copper (Barry 2011). These examples suggest that the impact of chemical stressors on the communication via infochemicals is diverse and highly substance- and species-specific.

Infochemicals may at the same time modify the effect induced by chemical stressors in aquatic ecosystems. Hanazato and Dodson (1995) reported synergistic effects on life-history parameters of *Daphnia pulex* caused by insect predator (*Chaoborus*) cues and the carbamate insecticide carbaryl (see, for fish cues, Oda et al. 2019). Similarly, *Ceriodaphnia dubia* showed a >70% lower survival in the presence of both the organophosphate insecticide malathion and predatory fish cues (*Pimephales promelas*) relative to malathion alone. This pattern was, however, not observed with another organophosphate insecticide, dicrotophos (Maul et al. 2006). Using *Ceriodaphnia* survival as a response variable and predatory fish cues (*Lepomis macrochirus*) as well as 5 different pesticides, Qin et al. (2011) documented synergistic effects for the broad-spectrum insecticide fipronil. For the neonicotinoid thiacloprid and the pyrethroid bifenthrin, Qin et al. (2011) reported antagonistic effects; and for malathion, they observed additivity. Pestana et al. (2010) assessed the interactive effects of predator and alarm cues (i.e., crushed *Daphnia*) with the neonicotinoid imidacloprid on life-history parameters of *D. magna*. These authors highlighted a significant interaction of both factors, likely caused by increased energy expenditure due to the neonicotinoid exposure. In another study, Pestana et al. (2009) did uncover mainly additive effects in *Chironomus riparius* and *Sericostoma vittatum* when exposed to fish cues (*Salmo trutta*) and imidacloprid. Similarly, *C. riparius* showed additive effects when jointly exposed to fish cues (*Cyprinus carpio*) and the organophosphate insecticide dimethoate (Van Praet et al. 2014). These studies highlight that, even within one taxonomic order (i.e., Cladocera), the joint effects of infochemicals (mainly predator cues) and pesticides are complex and seem to depend on the origin of the cues, the identity of the pesticide, as well as the test species. These complex interactions call for additional studies targeting the responses of different invertebrates to a joint exposure of infochemicals and chemical stressors to contribute—together with published data—to an ongoing debate on the impact of infochemicals.

We, therefore, assessed the impact of conspecific alarm cues (ground organisms) alone and in combination with the neonicotinoid insecticide thiacloprid on sublethal responses of the leaf-shredding amphipod *Gammarus fossarum*. This is a key species in the ecosystem function of leaf litter breakdown (Dangles et al. 2004) and acts as a predator of, for example, *Baetis* nymphs (Kelly et al. 2002a, 2002b). Consequently, the impacts on 2 top-down controlled ecosystem-level processes, namely leaf degradation and predation, were assessed using gammarid leaf consumption and their predation efficiency on *Baetis* spp. (Englert et al. 2012) as proxies. We hypothesized that conspecific alarm cues as well as thiacloprid will reduce gammarid feeding activity in a dose-dependent manner. Given the lack of a clear indication from the literature, we hypothesized further that alarm cues and thiacloprid will have additive effects on gammarid sublethal responses.

MATERIAL AND METHODS

Model toxicant

The model toxicant thiacloprid was applied as a commercially available formulation (Calypso® 480 SC; 480 g thiacloprid/L; Bayer CropScience), which rendered the use of further solvents unnecessary. The formulation was serially diluted in amphipod medium (SAM-5S Borgmann 1996) to receive the respective nominal concentrations in the range between 0.75 and 6 µg/L. To verify nominal concentrations at the start of the experiments, triplicate 10-mL samples were taken from the insecticide-free controls and the lowest thiacloprid treatments. Samples were stored at −20 °C until analysis via an ultra-high-performance liquid chromatography system as in our earlier work (Englert et al. 2017a). The analyses revealed adequate thiacloprid dosing for all experiments because nominal (0.75 and 1 µg/L) and measured initial (0.62 and 1.1 µg/L) concentrations deviated by <20%.

Conspecific alarm cues

Conspecific alarm cues were obtained by grinding 20 fresh *G. fossarum* of the same size as employed in the experiments using a glass mortar (Wisenden et al. 2001). The ground material was subsequently dispersed in SAM-5S (Borgmann 1996). Because cannibalism is common for gammarids (Dick 1995), the dispersion was filtered over a 0.5-mm mesh screen to remove larger particles that could serve as alternative food for gammarids and may thus interfere with the response variables assessed in the present study. Alarm cues were prepared and (re-)spiked daily during each experiment (see sections *Leaf consumption experiments* and *Predation experiments*) to ensure their constant availability and freshness.

Preparation of leaf discs

Leaf discs were prepared as described in Zubrod et al. (2010). Briefly, shortly before leaf fall in October 2012, black alder (*Alnus glutinosa* [L.] Gaertn.) leaves from trees in the vicinity of Landau, Germany (49°11′N, 8°05′E), were collected and stored at –20 °C until use. Discs of 2-cm diameter were cut from the leaves with a cork borer, while excluding the main vein. Leaf discs were subjected to microbial colonization (i.e., conditioning) for 10 d in a nutrient medium (Dang et al. 2005) using leaf material previously exposed in a near-natural stream (Rodenbach, Germany, 49°33′N, 8°02′E) as inoculum. After conditioning, leaf discs were dried to a constant weight (~24 h at 60 °C) and weighed to the nearest 0.01 mg. Approximately 48 h prior to the start of each experiment, leaf discs were resoaked in test medium (i.e., SAM-5S; Borgmann 1996) to prevent floating during the experiments.

Test organisms

Gammarus fossarum were kick-sampled in the near-natural stream Hainbach near Landau, Germany (49°14′N, 8°03′E, cryptic lineage B; Feckler et al. 2014) 7 d prior to each experiment. Gammarids were immediately divided into different size classes (Franke 1977), and only adult males—identified by their position in precopula pairs—with a cephalothorax length from 1.6 to 2.0 mm being visually free of acanthocephalan parasites were used to reduce variability in feeding behavior during the experiments (cf. Pascoe et al. 1995). Throughout the acclimation phase in the laboratory, animals were kept in aerated medium in a climate-controlled chamber at 20 ± 1 °C (for leaf consumption experiments) or 16 ± 1 °C (for predation experiments) in total darkness, while they were fed ad libitum with preconditioned black alder leaves and gradually adapted to SAM-5S (Borgmann 1996).

Baetis spp. nymphs were obtained from a near-natural stretch of the stream Triefenbach near Edenkoben, Germany (49°28′N, 8°09′E). Animals were collected 24 to 48 h prior to the start of each experiment by kick sampling. In the laboratory, mayfly nymphs were size-separated (selecting only animals of 7–10 mm length). Afterwards, animals were kept in aerated water from the sampling site at 16 ± 1 °C in total darkness, while algae-covered stones from the same site provided food.

Leaf consumption experiments

The general experimental design followed that of Zubrod et al. (2010). One *G. fossarum* was randomly placed together with 2 preconditioned leaf discs in a 250-mL glass beaker filled with 150 mL of SAM-5S containing either increasing levels of alarm cues or increasing concentrations of thiacloprid in the absence or presence of a defined (see below) alarm cue level. All beakers were aerated during the whole study duration. In addition to the replicates established to quantify gammarid leaf consumption, 5 replicates were set up per treatment without gammarids to account for the microbial and physical leaf mass loss. Experiments were run in total darkness at 20 ± 1 °C. Amphipods, the remaining leaf discs, and any leaf tissue shredded off were removed after 7 d of exposure, dried, and weighed as described in section *Preparation of leaf discs*.

The impact of increasing levels of alarm cues on gammarid feeding was assessed employing 6 treatments with a replication of 30 each. The treatment levels equaled 0, 0.11, 0.33, 1, 3, and 9 crushed gammarids over the whole study duration per replicate. Over the 7-d study, one-seventh of the total dose was respiked daily (by adding 7 mL/d), ensuring a constant availability of alarm cues (also simulating to some extent the constant exchange of water in streams). Based on the results of the present study, the intensity of alarm cues was selected for all follow-up studies as the highest level that did not lead to a statistically significant deviation in leaf consumption (measured as feeding rate) relative to the alarm cue–free control (i.e., one crushed gammarid over the whole study duration).

To judge the combined effect of thiacloprid and alarm cues on gammarid feeding rate, 6 levels of thiacloprid were selected (0, 1, 2, 2.5, 3, and 6 μg/L) centered around the concentration reported to reduce the feeding rate by 50% (median effect concentration [EC50]), namely between 1 and 4 μg/L (Feckler et al. 2012; Englert et al. 2017b). These thiacloprid concentrations were tested for their impact on gammarid leaf consumption in the presence and absence of alarm cues, leading to 12 treatments in total, which were replicated 25 times each. The level of alarm cues, as mentioned, did not significantly affect gammarid leaf consumption alone and equaled one crushed gammarid over the whole study duration. To ensure the constant availability of alarm cues also in this experiment, 7 mL of the alarm cue stock dispersion (containing also the respective thiacloprid concentration) was added to each replicate of each treatment daily. Similarly, 7 mL of SAM-5S (containing the respective thiacloprid concentration) were added daily to the alarm cue–free treatments.

Predation experiments

Two 2 × 2 factorial experiments were performed, assessing for the individual and combined impact of either 0.75 or 2 μg thiacloprid/L and alarm cues (one crushed gammarid over the whole study duration per replicate). Consequently, 4 treatments were realized in each experiment: an alarm cue–free and thiacloprid-free control, alarm cues only, thiacloprid only (at either 0.75 or 2 μg/L), and a combination of both alarm cues and thiacloprid. All treatments were replicated 16 times, and the experiment ran for 120 h at 16 ± 1 °C in darkness. The experimental design followed largely that of Englert et al. (2012) with some modifications: 8 *Beatis* spp. nymphs and 4 *G. fossarum* specimens were randomly placed together with 5 resoaked preweighed leaf discs in a crystallizing dish filled with 500 mL SAM-5S containing the respective levels of alarm cues and/or thiacloprid. Each treatment contained 2 additional replicates without animals to account for leaves' microbial and physical mass loss. To ensure a continuous availability of alarm cues in this experiment, the same approach was employed as for the experiments focusing on gammarid leaf consumption only. Briefly, 7 mL of the alarm cue stock dispersion or SAM-5S (without cues), both containing the respective thiacloprid concentration, were added to each replicate of each treatment daily.

The numbers of alive, dead, emerged, and consumed *Beatis* spp. nymphs were recorded every 12 h; and those nymphs that emerged were replaced. Mayfly nymphs were considered as consumed if <50% of their bodies remained. After the study duration of 120 h, all gammarids, remaining leaf discs and any visible leaf tissue shredded off were removed, dried at 60 °C to constant weight, and subsequently weighed to the nearest 0.01 mg. This procedure allowed us to assess, besides gammarid predation rate, their leaf consumption and body weight.

Calculations and statistics

The feeding rate on leaf discs was expressed in consumed leaf mass (C) and calculated as follows (Maltby et al. 2000):

$$C = \frac{(L_b \times k) - L_e}{g \times t} \tag{1}$$

where L_b represents initial dry mass of the leaf discs, L_e represents final dry mass of the leaf discs, g is the dry mass of

G. fossarum, *t* is feeding time in days, and *k* is leaf change correction factor given by:

$$k = \frac{\sum 1 - \left(\frac{L_{ob} - L_{oe}}{L_{ob}}\right)}{n} \tag{2}$$

where L_{ob} represents the initial dry mass of the leaf discs, L_{oe} represents the final dry mass of the leaf discs—both measured in replicates without any *G. fossarum* present—and *n* is the number of replicates.

The predation (*P*) of *G. fossarum* on mayflies was expressed as a percentage of consumed nymphs at each point in time:

$$P_t = \frac{Z_t}{N} \times 100 \tag{3}$$

where Z_t is the number of consumed nymphs at time *t* and *N* is the number of nymphs per treatment.

Because the feeding rate data of gammarids exposed to increasing levels of alarm cues did not meet the requirements for parametric testing (as judged by the Shapiro-Wilk test for normality), they were analyzed using the Kruskal-Wallis test. Subsequently, the Wilcoxon test with Bonferroni adjustment was employed to identify significant deviations between each treatment and the respective control. All other data sets were analyzed by 2-way analysis of variance (ANOVA) based on rank-transformed data where requirements for parametric testing were not met. The percentage of consumed mayflies (predation) over time was also assessed by 3-way ANOVA, with time being a fixed factor. The term "significant(ly)" is exclusively used in reference to statistical significance ($p < 0.05$) throughout the present study. For all statistics and figures, R, Ver 3.3.2 for Mac, was used.

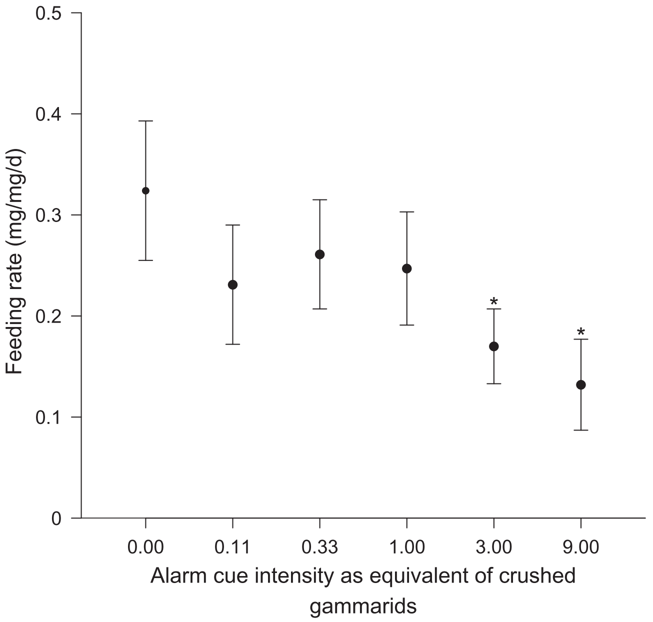

FIGURE 1: Mean (with 95% CI) feeding rate of *Gammarus fossarum* (mg leaf dry mass/mg *Gammarus* dry mass/d) exposed to increasing intensities of alarm cues expressed as equivalent of crushed gammarid filtrate added over a study duration of 7 d. *Significant differences compared to the control (Wilcoxon rank sum test, $p < 0.01$, $n = 27–30$).

RESULTS AND DISCUSSION

Impact of conspecific alarm cues

Infochemicals are central for the communication within and among species. This communication includes, besides pheromones that stimulate mating, also kairomones that shape predator–prey interactions. In this context, antipredator responses (morphological, behavioral, developmental, and physiological changes in prey) induced when sensing heterospecific predator cues (kairomones) released from vertebrates and invertebrates have attracted considerable attention over the last decades (Kats and Dill 1998; Paterson et al. 2013; reviewed in Jermacz and Kobak 2018). The impact of conspecific alarm substances, another set of infochemicals (Bronmark and Hansson 2000), is rarely assessed. We document, in agreement with our hypothesis, a reduction in the feeding rate of *G. fossarum* with increasing alarm cue intensity (Kruskal-Wallis, $p < 0.001$, $n = 27–30$; Figure 1). In the present study, alarm cues reduced gammarid feeding rate significantly (Wilcoxon rank sum test, $p = 0.009$, $n = 29–30$) by roughly 50% if their intensity equaled 3 or more crushed gammarids over 7 d. At lower intensities, the feeding rate of *G. fossarum* was reduced nonsignificantly by roughly 30%. Because indirect effects through changes in the leaf-associated microbial communities are unlikely to affect gammarid leaf consumption in this experimental setting (see for a detailed discussion Bundschuh and Schulz 2011), direct waterborne exposure is likely the most relevant effect pathway.

The alarm cue intensity–dependent decrease in leaf consumption reported in the present study contradicts the outcome of a meta-analysis on predator cues, which highlights that neither cue intensity nor exposure duration plays a significant role for antipredator responses (Paterson et al. 2013). It is, however, difficult to estimate how frequent the appearance of conspecific alarm cues at intensities directly affecting invertebrate behavior in aquatic ecosystems is, which calls for a quantitative characterization in the field. Moreover, invertebrates may become insensitive to these cues if present for a longer time period, questioning the relevant impact at the population level (Abjörnsson et al. 2009; Ahlgren et al. 2011). In the context of the present study, we did not intend to produce a substantial direct impact on sublethal response variables caused by alarm cues in the follow-up experiments. Hence, the highest alarm cue intensity not leading to a significant impact on the feeding rate was selected. This intensity equaled one crushed gammarid being added to the test medium of each replicate over the whole study duration, which is in the range of earlier studies (Pestana et al. 2009; Wisenden et al. 2009).

Joint effect of thiacloprid and conspecific alarm cues

Similar to ecological studies, the majority of publications assessing for consequences of chemical stressors on the

TABLE 1: Output table of factorial analysis of variances performed on rank-transformed or untransformed response data of gammarids from studies performed with thiacloprid at different concentrations and a fixed alarm cue intensity

	df	Sum of squares	Mean squares	*F*	*p*
Leaf consumption experiment					
Thiacloprid	5	692 497	138 499	31.413	<0.001
Alarm cues	1	36 583	36 583	8.178	0.005
Interaction	5	28 925	5785	1.312	0.259
Residuals	275	121 2451	4409		
Predation experiments (2 × 2 factorial design)					
0.75 μg/L thiacloprid and alarm cues					
Consumed nymphs (3-way ANOVA with time as random factor)					
Thiacloprid	1	0.515	0.5151	6.424	0.014
Alarm cues	1	0.730	0.7304	9.109	0.004
Interaction	1	0.041	0.0407	0.508	0.479
Residuals	56	4.491	0.0802		
Factor time					
Time	9	16.429	1.8254	227.749	<0.001
Time × thiacloprid	9	0.477	0.0529	6.606	<0.001
Time × alarm	9	0.104	0.0116	1.444	0.1660
Time × thiacloprid × alarm	9	0.138	0.0153	1.907	0.049
Residuals	509	4.080	0.0080		
Leaf consumption					
Thiacloprid	1	0.00	0.00	0.000	1.000
Alarm cues	1	5	4.51	0.016	0.901
Interaction	1	41	41.09	0.142	0.708
Residuals	53	15 382	290.23		
Gammarid weight					
Thiacloprid	1	21	21.5	0.088	0.768
Alarm cues	1	2433	2433.4	9.954	0.003
Interaction	1	15	14.9	0.061	0.806
Residuals	53	12 957	244.5		
2 μg/L thiacloprid and alarm cues					
Consumed nymphs (3-way ANOVA with time as random factor)					
Thiacloprid	1	5.645	5.645	56.760	<0.001
Alarm cues	1	0.020	0.020	0.198	0.658
Interaction	1	0.000	0.000	0.001	0.973
Residuals	60	5.967	0.099		
Factor time					
Time	9	24.621	2.7357	295.642	<0.001
Time × thiacloprid	9	5.840	0.6489	70.130	<0.001
Time × alarm	9	0.066	0.0073	0.789	0.627
Time × thiacloprid × alarm	9	0.047	0.0052	0.562	0.829
Residuals	551	5.099	0.0093		
Leaf consumption					
Thiacloprid	1	6037	6037	25.882	<0.001
Alarm cues	1	148	148	0.636	0.428
Interaction	1	886	886	3.797	0.056
Residuals	59	13 761	233		
Gammarid weight					
Thiacloprid	1	3	2.7	0.008	0.931
Alarm cues	1	2	1.9	0.005	0.942
Interaction	1	29	29.0	0.082	0.775
Residuals	59	20 797	352.5		

communication through infochemicals (Lurling and Scheffer 2007) and those addressing the impact of infochemicals on chemical stressor toxicity (see *Introduction*) have involved predator cues (but see Pestana et al. 2009). We document that conspecific alarm cues have a small but consistent and significant (Table 1 and Figure 2) effect on gammarid feeding when applied jointly with thiacloprid. The largely additive impact of alarm cues resulted, at 2 and 3 μg thiacloprid/L, in significantly reduced feeding rates in their presence relative to their absence ($p < 0.05$; effect size was 30 and 40%, respectively). At higher thiacloprid concentrations (i.e., 6 μg/L) the impact of alarm cues was limited, which may be explained by the overriding impact of thiacloprid because this concentration is at least 2-fold above the EC50 for the same variable, as recalculated from an earlier study (Feckler et al. 2012). The additivity of effects induced by a combined exposure to alarm cues and pesticides may, however, not be transferable to pesticides from the same or other substance classes, as shown

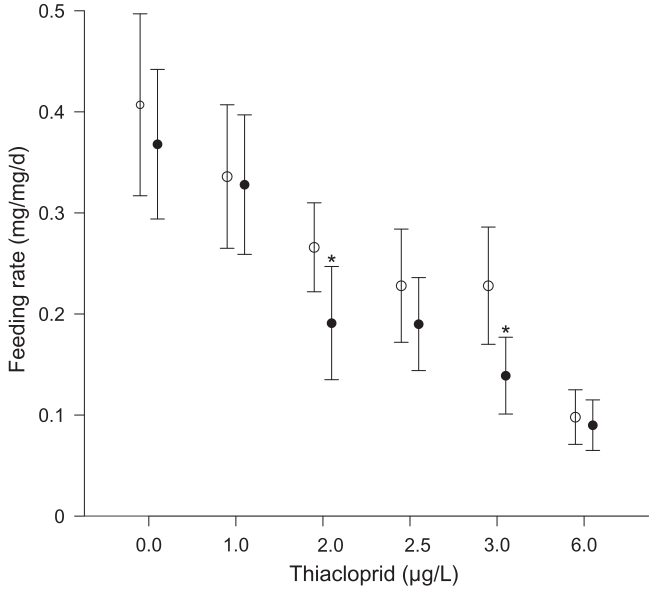

FIGURE 2: Mean (with 95% CI) feeding rate of *Gammarus fossarum* (mg leaf dry mass/mg *Gammarus* dry mass/d) exposed to increasing concentrations of thiacloprid in the absence (open symbols) and presence of alarm cues (solid symbols). The latter were applied at an equivalent of one crushed gammarid filtrate over the study duration of 7 d. *Significant differences between the presence and absence of alarm cues at the same thiacloprid concentration (Wilcoxon rank sum test, $p < 0.05$, $n = 23–25$).

for *Ceriodaphnia dubia* by Maul et al. (2006) and Qin et al. (2011). This assumption is also supported by a side experiment with a fungicide suggesting no impact of conspecific alarm cues on tebuconazole-induced effects (Supplemental Data, Figure S1). Tebuconazole and thiacloprid have a comparable organic–carbon partition coefficient (~700, Lewis et al. 2016), making differences in their bioavailability unlikely, and thus this cannot explain discrepancies in the observed alarm cue impact (but see Qin et al. 2011).

Based on the feeding rate data (Figure 2), 2 thiacloprid concentrations were selected to assess gammarid predation on the nymphs of *Baetis* spp. as well as the gammarid weight and leaf consumption in the presence and absence of conspecific alarm cues. The low concentration (0.75 µg/L) was selected, assuming limited direct effects of thiacloprid on *Gammarus*, whereas the high concentration (2 µg/L) was expected to lead to direct effects in all assessed variables (Englert et al. 2012).

Alarm cues and 0.75 µg/L thiacloprid affected the proportion of *Baetis* nymphs preyed on by gammarids. Thiacloprid led to consistently higher predation by gammarids (see also Englert et al. 2012) being (according to 2-way ANOVA) statistically significant from 72 h after test initiation onward. This observation is likely driven by the higher sensitivity to thiacloprid of *Baetis* relative to *Gammarus* and their inability to escape predation (see, for details, Englert et al. 2012). The presence of alarm cues reduced predation significantly over the study duration (Table 1 and Figure 3A) through either conspecific or heterospecific effects on gammarids or mayfly nymphs, respectively; 2-way ANOVAs performed for each monitoring time point highlight this factor as significant between 48 and 96 h after test start (Figure 3A). This variation in factors triggering significant effects in gammarid predation suggests that the impact of alarm cues is overridden by thiacloprid with increasing exposure duration (see also the significant interaction term of the factors time, thiacloprid, and alarm cues). The decreased predation caused by alarm cues may be triggered by a lower locomotion of gammarids, as observed by Åbjörnsson et al. (2000), and a shorter period of time spent on prey consumption (Iacarella et al. 2018). In contrast to gammarid predation, leaf consumption remained stable over the treatments (Figure 3B). This observation is in agreement with the present study (Figure 1) and that of Feckler et al. (2012), reporting limited effects in this variable at similar alarm cue intensities and thiacloprid concentrations. The dry weight of gammarids, however, was, with an effect size <10%, significantly reduced when experiencing alarm cue exposure (Table 1 and Figure 3C), suggesting—though not assessed in the present study—a severe impact on growth rate over the study duration of only 120 h. These data suggest that, irrespective of the success in preying on mayfly nymphs, gammarids did not compensate by increasing leaf consumption. At the same time, the higher predation in the thiacloprid treatment did not increase gammarid growth relative to the control. Such a pattern might indicate that internal detoxification and defense mechanisms required the additional energy available from mayfly predation (Rasmussen et al. 2017). In contrast, gammarids in both treatments with alarm cues showed a low but significant decrease in animal dry weight. Hence, animal dry weight seemed to reflect the lower ingestion of high-quality food, namely insect nymphs.

This alarm cue–induced reduction in gammarid dry weight together with their lower efficiency to prey was not confirmed in the second experiment (Table 1 and Figure 4A and C). Similarly, thiacloprid did not affect this variable, which is unexpected as predation was significantly positively affected in the presence of 2 µg thiacloprid/L (Table 1 and Figure 4A), a pattern that led to a meaningful increase in dry weight in earlier studies (Englert et al. 2012). It can be assumed that the increased success in predation by gammarids, which led to a higher consumption of high-quality food was—according to the dynamic energy budget theory (Koojiman 2000)—allocated to other physiological processes. It is likely that gammarids, when exposed to 2 µg thiacloprid/L, favored the investment in maintenance, namely detoxification, repair, and defense mechanisms (Maltby 1999; Rasmussen et al. 2017), over growth (sensu Naylor et al. 1989). The presumed higher energy demand was also not balanced by an elevated leaf consumption (Table 1 and Figure 4B). Leaf consumption was in fact significantly negatively affected by thiacloprid, with a nearly significant interaction between thiacloprid and alarm cues (Table 1 and Figure 4B). This nearly significant interaction term confirms the insights from the feeding rate study (Figure 2) by indicating an impact of alarm cues on this parameter at thiacloprid concentrations in the low µg/L range.

Overall, the impact of alarm cues on gammarids was inconsistent among the 2 predation experiments, which kept the

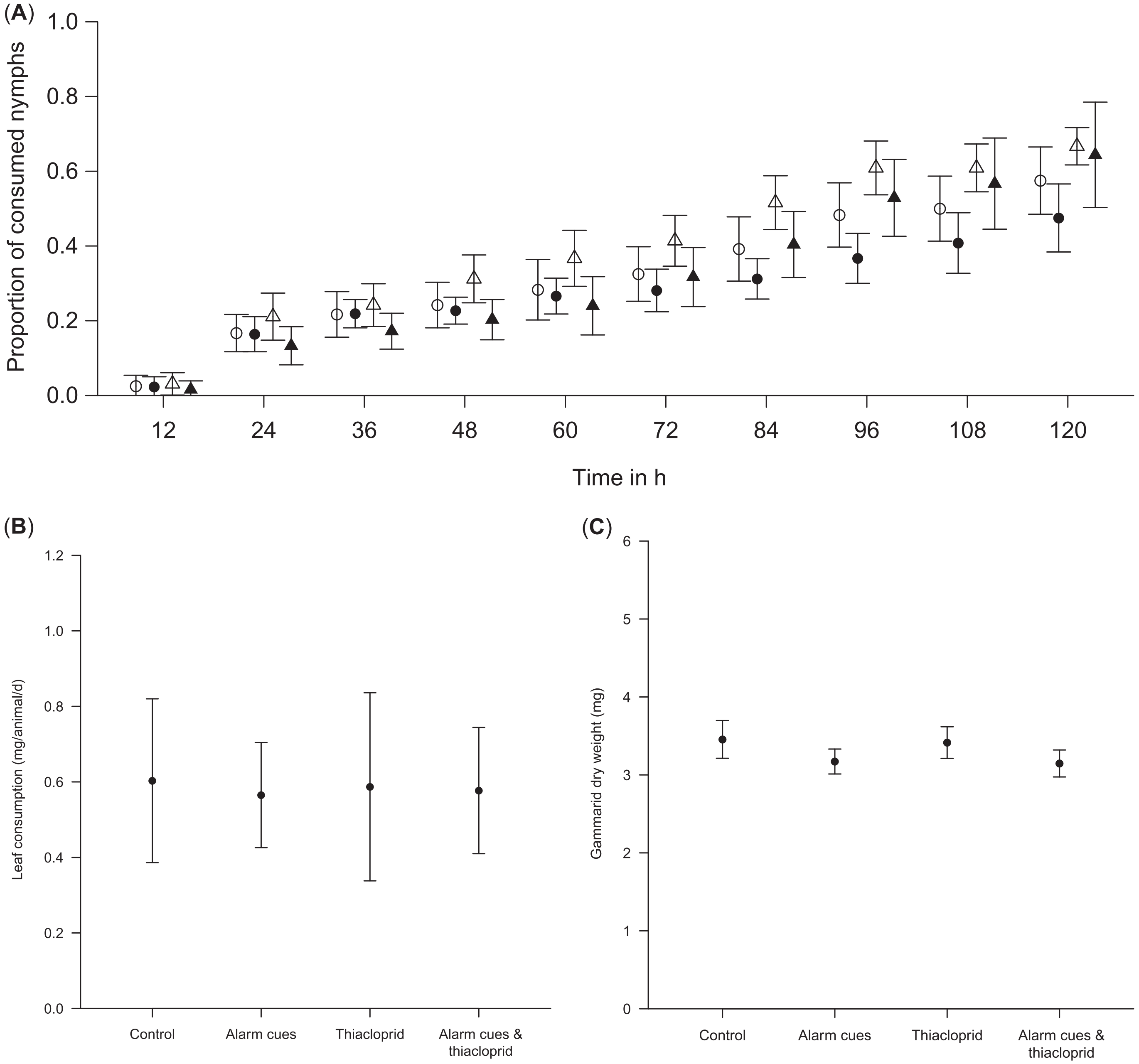

FIGURE 3: (**A**) Development of the mean (with 95% CI) proportion of consumed mayfly nymphs by *Gammarus fossarum* over 120 h in the absence (circles) and presence (triangles) of 0.75 μg thiacloprid/L and in the absence (open symbols) and presence (filled symbols) of alarm cues. (**B**) Mean (with 95% CI) consumed leaf mass per gammarid. (**C**) Gammarid dry weight at the termination of the experiment.

conspecific alarm cue intensity constant but varied thiacloprid concentrations. This discrepancy may be explained by a much less pronounced impact of alarm cues, when applied alone, in the second relative to the first experiment (Figures 3A and 4A). It is, therefore, more likely that conspecific alarm cues vary in their availability or effect over time (see also Smith and Webster 2015); this can be driven by either a variable availability of alarm cues in gammarids, efficiency in setting the cues free during grinding, stability of alarm cues (Wisenden et al. 2009), or susceptibility of the test organisms toward those cues over seasons and developmental stages (sensu Meuthen et al. 2019). Indeed, the time lag between experiments (a few months) suggests changes in their energy reserves (Becker et al. 2013), which could translate to a shift in sensitivity (Prato and Blandolino 2009) as conceptualized in the dynamic energy budget theory (Kooijman 2000). Nonetheless and in accordance with our second hypothesis, the present study suggests that alarm cues can increase thiacloprid-induced sublethal effects in aquatic invertebrates, with their interactions being mainly additive. Hence, the present study contributes to an ongoing debate on the impact of infochemicals on chemical stress–induced effects in aquatic ecosystems by highlighting

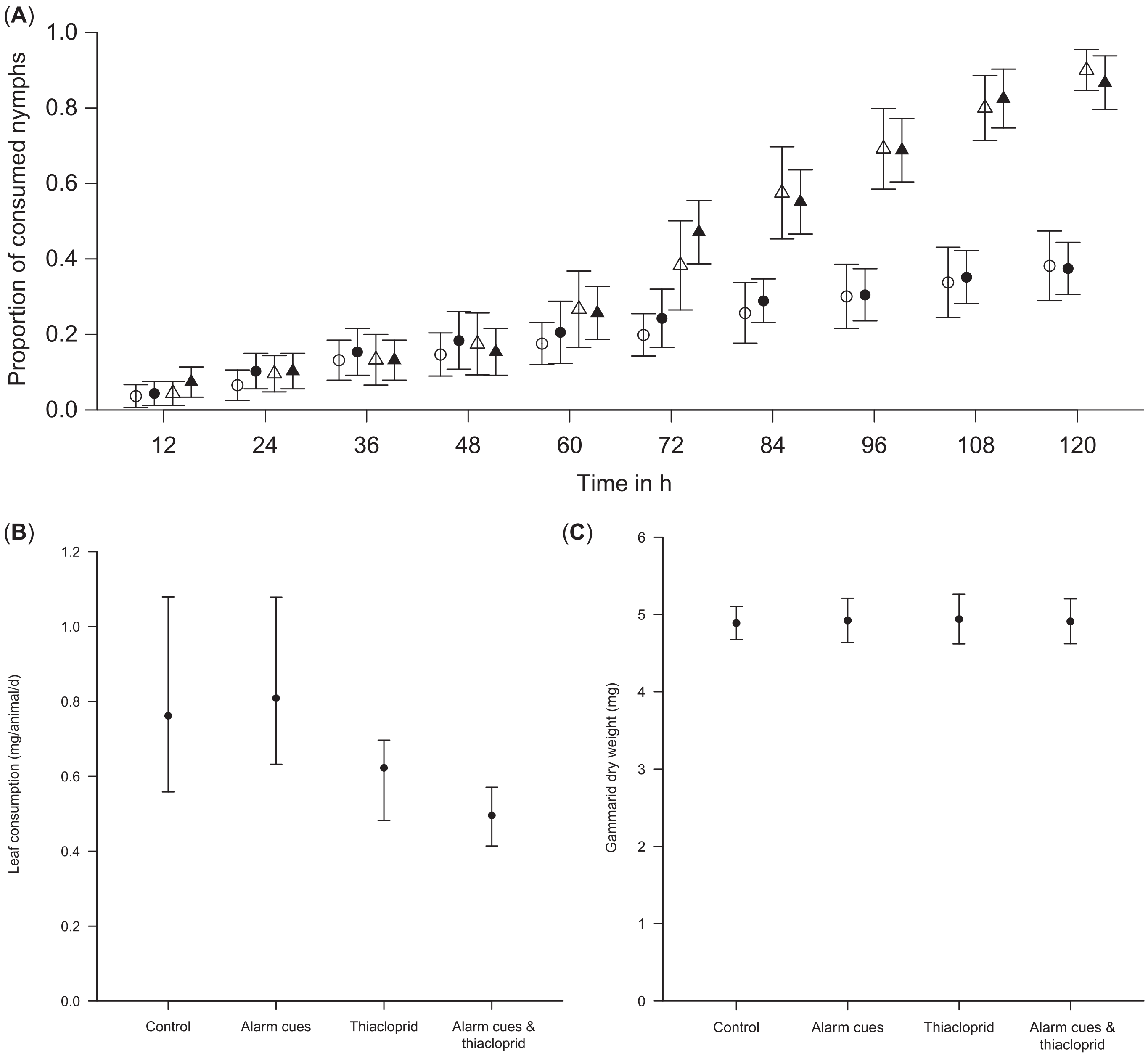

FIGURE 4: (**A**) Development of the mean (with 95% CI) proportion of consumed mayfly nymphs by *Gammarus fossarum* over 120 h in the absence (circles) and presence (triangles) of 2 µg thiacloprid/L and in the absence (open symbols) and presence (filled symbols) of alarm cues. (**B**) Mean (with 95% CI) consumed leaf mass per gammarid. (**C**) Gammarid dry weight at the termination of the experiment.

the context-dependent interaction (e.g., Maul et al. 2006; Qin et al. 2011).

CONCLUSION

The present study highlights that conspecific alarm cues show additive effects when present with the neonicotinoid insecticide thiacloprid on sublethal responses of the amphipod *G. fossarum*. Whereas alarm cues increase the negative effect of thiacloprid in gammarid leaf consumption, they buffered the increased predation of this species on mayfly nymphs at a low thiacloprid concentration. These alterations may have important consequences for the ecosystem function of leaf litter decomposition and for the trophic interactions within exposed communities. The latter could lead to a reduced predation pressure on lower trophic levels under chemical stress. The variable impacts of alarm cues on thiacloprid-induced effects and those caused by the other pesticide, tebuconazole (as shown in the side experiment; Supplemental Data, Figure S1), highlight that either the test species react differently to alarm cues or the quality of cues varies across seasons or developmental stages. To further advance the mechanistic insights in this complex interplay of multiple factors on the behavior of invertebrates, future efforts should target these fundamental aspects. This would also further our ability to predict effects in a multiple stressor framework.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4802.

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Data Availability Statement—The data and R-code are available on request from the corresponding author (bundschuh@uni-landau.de).

REFERENCES

Abjörnsson K, Bronmark C, Hansson LA. 2009. The influence of predator regime on reproductive traits in *Gammarus pulex* populations. *Hydrobiologia* 635:215–225.

Åbjörnsson K, Dahl J, Nyström P, Brönmark C. 2000. Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Aquat Ecol* 34:379–387.

Ahlgren J, Abjörnsson K, Brönmark C. 2011. The influence of predator regime on the behaviour and mortality of a freshwater amphipod, *Gammarus pulex*. *Hydrobiologia* 671:39–49.

Barry MJ. 1998. Endosulfan-enhanced crest induction in *Daphnia longicephala*: Evidence for cholinergic innervation of kairomone receptors. *J Plankton Res* 20:1219–1231.

Barry MJ. 2011. Effects of copper, zinc and dragonfly kairomone on growth rate and induced morphology of *Bufo arabicus* tadpoles. *Ecotoxicol Environ Saf* 74:918–923.

Bayram A, Salerno G, Onofri A, Conti E. 2010. Sub-lethal effects of two pyrethroids on biological parameters and behavioral responses to host cues in the egg parasitoid *Telenomus busseolae*. *Biol Control* 53:153–160.

Becker J, Ortmann C, Wetzel MA, Winkelmann C, Koop JHE. 2013. Mate guarding in relation to seasonal changes in the energy reserves of two freshwater amphipods (*Gammarus fossarum* and *G. pulex*). *Freshw Biol* 58:372–381.

Borgmann U. 1996. Systematic analysis of aqueous ion requirements of *Hyalella azteca*: A standard artificial medium including the essential bromide ion. *Arch Environ Contam Toxicol* 30:356–363.

Bronmark C, Hansson LA. 2000. Chemical communication in aquatic systems: An introduction. *Oikos* 88:103–109.

Bundschuh M, Schulz R. 2011. Ozonation of secondary treated wastewater reduces ecotoxicity to *Gammarus fossarum* (Crustacea; Amphipoda): Are loads of (micro)pollutants responsible? *Water Res* 45:3999–4007.

Dang CK, Chauvet E, Gessner MO. 2005. Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. *Ecol Lett* 8:1129–1137.

Dangles O, Gessner MO, Guerold F, Chauvet E. 2004. Impacts of stream acidification on litter breakdown: Implications for assessing ecosystem functioning. *J Appl Ecol* 41:365–378.

Delpuech JM, Dupont C, Allemand R. 2012. Effects of deltamethrin on the specific discrimination of sex pheromones in two sympatric trichogramma species. *Ecotoxicol Environ Saf* 84:32–38.

Dick JTA. 1995. The cannibalistic behaviour of two *Gammarus* species (Crustacea: Amphipoda). *J Zool* 236:697–706.

Dicke M, Takken W, eds. 2006. *Chemical Ecology—From Gene to Ecosystem*. Springer, Dordrecht, The Netherlands.

Englert D, Bakanov N, Zubrod JP, Schulz R, Bundschuh M. 2017a. Modeling re-mobilization of neonicotinoid residues from tree foliage in streams—A relevant exposure pathway in risk assessment? *Environ Sci Technol* 51:1785–1794.

Englert D, Bundschuh M, Schulz R. 2012. Thiacloprid affects trophic interaction between gammarids and mayflies. *Environ Pollut* 167:41–46.

Englert D, Zubrod JP, Link M, Mertins S, Schulz R, Bundschuh M. 2017b. Does waterborne exposure explain effects caused by neonicotinoid-contaminated plant material in aquatic systems? *Environ Sci Technol* 51:5793–5802.

Feckler A, Thielsch A, Schwenk K, Schulz R, Bundschuh M. 2012. Differences in the sensitivity among cryptic lineages of the *Gammarus fossarum* complex. *Sci Total Environ* 439:158–164.

Feckler A, Zubrod JP, Thielsch A, Schwenk K, Schulz R, Bundschuh M. 2014. Cryptic species diversity: A disregarded factor in environmental management? *J Appl Ecol* 51:958–967.

Franke U. 1977. Experimentelle untersuchungen zur respiration von *Gammarus fossarum* in abhängigkeit von temperatur, sauerstoffkonzentration und wasserbewegung. *Archiv für Hydrobiologie Supp* 3/4:369–411.

Hanazato T. 1991. Effects of repeated application of carbaryl on zooplankton communities in experimental ponds with or without the predator *Chaoborus*. *Environ Pollut* 74:309–324.

Hanazato T, Dodson SI. 1995. Synergistic effects of low oxygen concentration, predator kairomone, and a pesticide on the cladoceran *Daphnia pulex*. *Limnol Oceanogr* 40:700–709.

Iacarella JC, Hudgins EJ, Dick JTA, Ricciardi A. 2018. Predatory behaviour of an invasive amphipod in response to varying conspecific densities under higher-order predation risk. *Can J Fish Aquat Sci* 75:131–140.

Jermacz L, Kobak J. 2018. The braveheart amphipod: A review of responses of invasive *Dikerogammarus villosus* to predation signals. *PeerJ* 6:e5311.

Kats LB, Dill LM. 1998. The scent of death: Chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361–394.

Kelly DW, Dick JTA, Montgomery WI. 2002a. Predation on mayfly nymph, *Baetis rhodani*, by native and introduced *Gammarus*: Direct effects and the faciliation of predation by salmonids. *Freshw Biol* 47:1257–1268.

Kelly DW, Dick JTA, Montgomery WI. 2002b. The functional role of *Gammarus* (Crustacea, Amphipoda): Shredder predator, or both? *Hydrobiologia* 485:199–203.

Kooijman SALM. 2000. *Dynamic Energy and Mass Budgets in Biological Systems*. Cambridge University, Cambridge, UK.

Lewis KA, Tzilivakis J, Warner D, Green A. 2016. An international database for pesticide risk assessments and management. *Hum Ecol Risk Assess* 22:1050–1064.

Lurling M, Scheffer M. 2007. Info-disruption: Pollution and the transfer of chemical information between organisms. *Trends Ecol Evol* 22:374–379.

Maltby L. 1999. Studying stress: The importance of organism-level responses. *Ecol Appl* 9:431–440.

Maltby L, Clayton SA, Yu H, McLoughlin N, Wood RM, Yin D. 2000. Using single-species toxicity tests, community-level response and toxicity identification evaluations to investigate effluent impacts. *Environ Toxicol Chem* 19:151–157.

Maul JD, Farris JL, Lydy MJ. 2006. Interaction of chemical cues from fish tissues and organophosphorous pesticides on *Ceriodaphnia dubia* survival. *Environ Pollut* 141:90–97.

Meuthen D, Ferrari MCO, Lane T, Chivers DP. 2019. Predation risk induces age- and sex-specific morphological plastic responses in the fathead minnow *Pimephales promelas*. *Sci Rep* 9:15378.

Naylor C, Maltby L, Calow P. 1989. Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. *Hydrobiologia* 188/189:517–523.

Oda Y, Sato K, Hanazato T, Chang KH, Sakamoto M. 2019. Enhanced sensitivity to an insecticide carbaryl in *Daphnia magna* mediated by fish kairomone. *Limnology* 20:137–141.

Pascoe D, Kedwards TJ, Blockwell SJ, Taylor EJ. 1995. *Gammarus pulex* (L.) feeding bioassay—Effects of parasitism. *Bull Environ Contam Toxicol* 55:629–632.

Paterson RA, Pritchard DW, Dick JTA, Alexander ME, Hatcher MJ, Dunn AM. 2013. Predator cue studies reveal strong trait-mediated effects in communities despite variation in experimental designs. *Anim Behav* 86:1301–1313.

Pestana JLT, Loureiro S, Baird DJ, Soares AMVM. 2009. Fear and loathing in the benthos: Responses of aquatic insect larvae to the pesticide imidacloprid in the presence of chemical signals of predation risk. *Aquat Toxicol* 93:138–149.

Pestana JLT, Loureiro S, Baird DJ, Soares AMVM. 2010. Pesticide exposure and inducible antipredator responses in the zooplankton grazer, *Daphnia magna* straus. *Chemosphere* 78:241–248.

Petrusek A, Tollrian R, Schwenk K, Haas A, Laforsch C. 2009. A “crown of thorns” is an inducible defense that protects *Daphnia* against an ancient predator. *Proc Natl Acad Sci USA* 106:2248–2252.

Prato E, Blandolino F. 2009. Factors influencing the sensitivity of *Gammarus aequicauda* population from Mar Piccolo in Taranto (southern italy). *Ecotoxicol Environ Saf* 72:770–774.

Qin GQ, Presley SM, Anderson TA, Gao WM, Maul JD. 2011. Effects of predator cues on pesticide toxicity: Toward an understanding of the mechanism of the interaction. *Environ Toxicol Chem* 30: 1926–1934.

Rasmussen JJ, Reiber L, Holmstrup M, Liess M. 2017. Realistic pesticide exposure through water and food amplifies long-term effects in a limnephilid caddisfly. *Sci Total Environ* 580:1439–1445.

Smith LA, Webster MM. 2015. *Gammarus pulex* show a grouping response to conspecific injury cues but not to predator kairomones. *Behav Ecol* 26:1188–1195.

Van Praet N, De Jonge M, Stoks R, Bervoets L. 2014. Additive effects of predator cues and dimethoate on different levels of biological organisation in the non-biting midge *Chironomus riparius*. *Aquat Toxicol* 155:236–243.

Wisenden BD, Pohlman SG, Watkin EE. 2001. Avoidance of conspecific injury-released chemical cues by free-ranging *Gammarus lacustris* (Crustacea:Amphipoda). *J Chem Ecol* 27:1249–1258.

Wisenden BD, Rugg ML, Korpi NL, Fuselier LC. 2009. Lab and field estimates of active time of chemical alarm cues of a cyprinid fish and an amphipod crustacean. *Behaviour* 146:1423–1442.

Zubrod JP, Bundschuh M, Schulz R. 2010. Effects of subchronic fungicide exposure on the energy processing of *Gammarus fossarum* (Crustacea; Amphipoda). *Ecotoxicol Environ Saf* 73:1674–1680.