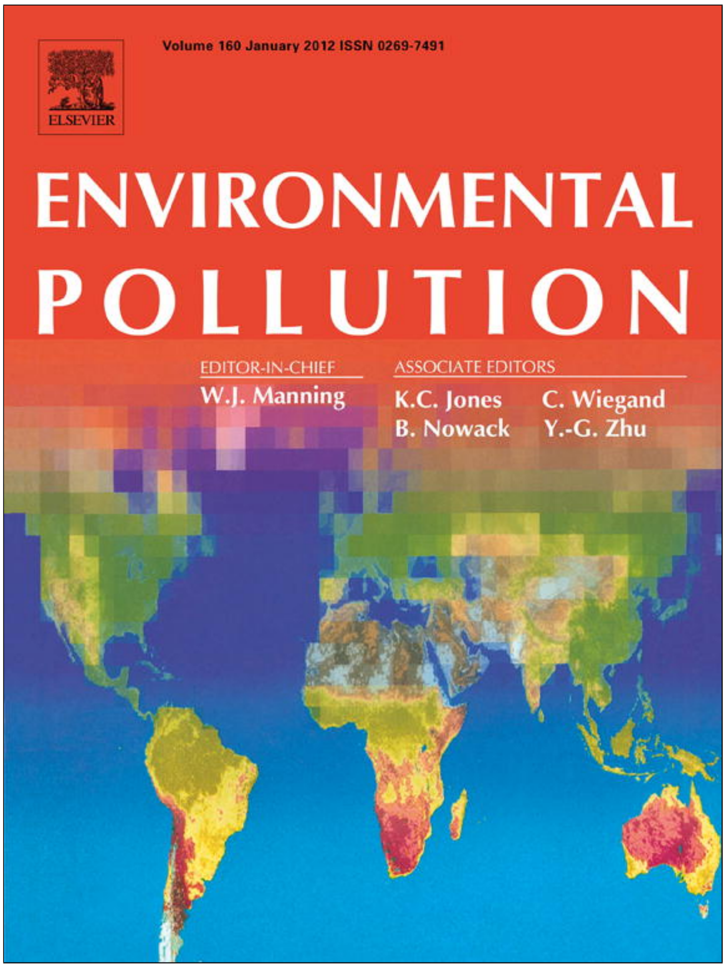
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Thiacloprid affects trophic interaction between gammarids and mayflies

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ABSTRACT

Neonicotinoid insecticides like thiacloprid enter agricultural surface waters, where they may affect predator–prey-interactions, which are of central importance for ecosystems as well as the functions these systems provide. The effects of field relevant thiacloprid concentrations on the leaf consumption of *Gammarus fossarum* (Amphipoda) were assessed over 96 h ($n = 13–17$) in conjunction with its predation on *Baetis rhodani* (Ephemeroptera) nymphs. The predation by *Gammarus* increased significantly at 0.50–1.00 μg/L. Simultaneously, its leaf consumption decreased with increasing thiacloprid concentration. As a consequence of the increased predation at 1.00 μg/L, gammarids' dry weight rose significantly by 15% compared to the control. At 4.00 μg/L, the reduced leaf consumption was not compensated by an increase in predation causing a significantly reduced dry weight of *Gammarus* (~20%). These results may finally suggest that thiacloprid adversely affects trophic interactions, potentially translating into alterations in ecosystem functions, like leaf litter breakdown and aquatic-terrestrial subsidies.

1. Introduction

Insecticides enter aquatic ecosystems through a variety of point and non-point sources (Carter, 2000). Particularly in intensively cultivated catchments, inputs by spray drift, direct spraying or field runoff following heavy precipitation result in transient insecticide contamination of streams (Schulz, 2004). Accordingly, the widely used neonicotinoid thiacloprid, which binds selectively to the nicotinic acetylcholine receptors of insects (Jeschke et al., 2001), potentially occurs in freshwaters (Beketov and Liess, 2008a). Its predicted surface water concentrations, calculated based on worst case assumptions (spray drift as the main route of entry, estimated on the basis of the maximum application rate), range from 2.0 to 17.5 μg/L for ornamentals and orchards, respectively (Schmuck, 2001). Moreover, thiacloprid was already measured at concentrations up to 4.5 μg/L in ditches of the orchard region "Altes Land", Germany (Süß et al., 2006). Although seasonal variation in the surface water concentrations of thiacloprid are not available in literature, it may be assumed that this substance is present through the year, as indicated by Tennekes (2010) for imidacloprid, another neonicotinoid insecticide.

Even though the 48 h-EC_{50} of thiacloprid for the standard test organism *Daphnia magna* (Crustacea) is at 85.1 mg/L (FOOTPRINT, 2011), a risk for aquatic ecosystems may be expected as other publications report 96 h-LC_{50} values, especially for insect nymphs, in the low μg/L-range (Beketov and Liess, 2008b). Moreover, sublethal behavioral endpoints, like organismic drift, are affected at concentrations even below those causing acute toxicity (Beketov and Liess, 2008b). However, such single species toxicity tests as well as higher tier mesocosm studies have only limited capability to provide sufficient information for the understanding and assessment of potential adverse interaction effects since important processes might be masked by other factors or are simply not displayed in required resolution (Schulz and Liess, 2001). Hence, potential effects of chemical stressors on the interaction among species of different trophic levels, such as predator–prey-interactions (e.g. Schulz and Dabrowski, 2001; Pestana et al., 2009a; Junges et al., 2010; Bundschuh et al., in press), need to be further investigated to reliably predict and understand effects at the ecosystem level.

In agricultural surface waters of central Europe two co-occurring invertebrate genera, namely *Gammarus* (Crustacea; Amphipoda) and *Baetis* (Insecta; Ephemeroptera) (e.g. Habdija, 1986; Bauernfeind and Moog, 2000), form an essential part of food webs as they act as prey for fish and invertebrates (e.g. Brittain, 1982; MacNeil et al., 1999). While, gammarids reproduce throughout the year (Pöckl, 1993) and hence reach abundances of up to several 1000 individuals per square meter (Ladewig et al., 2006), *Baetis* mayflies are mainly bivoltine. They spend the magnitude of their life as eggs and nymphs submerged (Brittain,

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1982). By emerging *Baetis* transport energy across ecosystem boundaries, from aquatic to terrestrial ecosystems (Jackson and Fisher, 1986). Moreover, *Gammarus fossarum* is known as a key-species in the ecosystem function of leaf litter breakdown (Dangles et al., 2004; Piscart et al., 2009, 2011). In addition it acts as a predator, which preys e.g. upon *Baetis* nymphs (Kelly et al., 2002a, 2002b; Felten et al., 2008). Hence, an insecticide driven alteration in the interaction between these two groups may affect the energy transfer both within surface waters and across ecosystem boundaries (Dewey, 1986; Peckarsky et al., 1993, 2001; Alexander et al., 2008; Pestana et al., 2009a). The present study therefore, assessed implications in the predator–prey-interactions due to thiacloprid exposure for 96 h at field relevant concentrations (0.50, 0.75, 1.00 and 4.00 μg/L). Based on both, the 96-h LC_{50} values for *Baetis rhodani* and *G. pulex* (4.6 and 350 μg thiacloprid/L respectively; Beketov and Liess, 2008b) as well as the higher sensitivity of insects toward neonicotinoids compared to crustaceans (Song et al., 1997; Stenersen, 2004; Beketov and Liess, 2008b), sublethal effects on *Baetis* were expected to increase *G. fossarums'* efficiency to prey upon the mayflies. As food quality and quantity are driving factors in terms of growth and reproduction (Delong et al., 1993; Gergs and Rothhaupt, 2008), alterations in *G. fossarums'* leaf consumption, its predation upon mayfly nymphs and final dry weight were determined.

2. Material & methods

2.1. Chemicals

Thiacloprid belongs to the group of neonicotinoids, a relatively new group of systemic insecticide with obviously high water solubility of 185 mg/L at 20 °C (Jeschke et al., 2001). Thiacloprid was applied using the commercial formulation Biscaya® (Bayer CropScience Limited, Germany; additives: ethoxylated alcohol and 2-ethylhexanol propylene ethyleneglycol ether) containing 240 g active ingredient/L. The formulation – instead of the pure active ingredient – was used since thiacloprid is applied as formulation and may thus enter the environment together with its additives. To obtain stock solutions with a nominal concentration of 5.00 g thiacloprid/L, Biscaya® was diluted in stream water (Hainbach, 49°14′N; 8°03′E) prior to each of the four independent experiments. Subsequently, nominal thiacloprid concentrations of 0.50, 0.75, 1.00 or 4.00 μg active ingredient/L (one concentration per experiment) were prepared by further serial dilution with stream water. Water quality parameters of the stream water were measured prior to the experiment with a WTW Multi 340i/SET (Wissenschaftlich Technische Werkstätten GmbH, Weilheim, Germany) as well as Macherey–Nagel visocolor® kits (Macherey–Nagel, Düren, Germany) (Table 1).

For thiacloprid analysis, 15 mL ($n = 3$) of test media were sampled from test vessels from both thiacloprid treatment and the corresponding control at the start of the respective experiments and stored frozen at −20 °C until further use. Chemical analyses were performed using an ultra HPLC mass spectrometry (UHPLC-MS) system equipped with an EQuan MAX system. The UHPLC-MS consists of a Combipal autosampler (CTC Analytics, Zwingen, Switzerland), an Accela Pump, a Hypersil Gold C18 column (50 × 2.1 mm, particle size 1.9 μm) and an Exactive system (if not mentioned otherwise from Thermo Fisher Scientific, Dreieich, Germany). Eluents A and B were water-purified with a MilliQ system (Millipore, Schwalbach, Germany) and methanol (LC-MS grade, Merck, Darmstadt, Germany), respectively, both containing 4 mM ammonium format and 0.1% formic acid (both Sigma Aldrich, Seelze, Germany, p.a. grade). After injection of 0.5 mL, the sample was pre-concentrated on the trap column (EQuan MAX system with a Surveyor pump and a Hypersil Gold aQ column; 20 × 2.1 mm, particle size 12 μm) with a flow rate of 2.0 mL/min of 98% A and 2% B. The trap column was back-flushed 2.0 min after injection with a flow rate of 0.1 mL/min to transfer the analytes online to the analytical column for chromatographic separation. Starting 4.0 min after injection, the analytical column was run isocratically for 4.5 min at 0% A and 100% B with a flow rate of 0.2 mL/min before rinsing with 5% A and 95% B for 1.5 min. Nitrogen was used as sheath and auxiliary gas with flow rates of 20.0 mL/min and 5.0 mL/min, respectively. Thiacloprid was ionized using an Ion Max API source with an ESI probe with a spray voltage of 2.2 kV and was quantified in the positive mode at the accurate mass of m/z 253.03 $[M + H^+]$. External calibration ranging from 0.375 to 12.00 μg/L was used. As mean measured thiacloprid concentrations (Table 2) were within 20% of the nominal concentrations, the latter is reported throughout the present study.

Table 1
Water quality parameters of the river water from the Hainbach (mean ± 95% CI; $n = 3$), measured prior to the start of the experiments.

Parameter	Value
pH	7.4 (± 0.1)
Conductivity (μS/cm)	134 (± 2)
Nitrite (mg/L)	0.002 (± 0.001)
Nitrate (mg/L)	2.3 (± 1.2)
Ammonium (mg/L)	<0.02
Oxygen saturation (%)	99.2 (± 0.1)

2.2. Preparation of leaf discs

Preparation of leaf discs followed the procedure described in detail by Bundschuh et al. (2011b). Briefly, discs of 2.0 cm diameter were cut from frozen (−20 °C) black alder leaves (*Alnus glutinosa* (L.) Gaertn.) collected shortly before leaf fall in October 2008 from trees near Landau, Germany (49°11′N; 8°05′E). The leaf discs were conditioned in a nutrient medium (Dang et al., 2005) for 10 d together with black alder leaves previously exposed in the Rodenbach stream, Germany (49°33′N, 8°02′E), to establish a natural microbial community consisting of fungi and bacteria. This conditioning process increases the nutritive value of leaf material for shredders, such as gammarids (Bärlocher, 1985), and simulates the environmentally relevant processes. After conditioning, the leaf discs were dried at 60 °C, weighed to the nearest 0.01 mg and re-soaked in stream water from the Hainbach for 24 h prior to the start of each experiment.

2.3. Test organisms

Both test species, *G. fossarum* and *B. rhodani*, originated from the Hainbach, a near natural stream. *G. fossarum* were collected upstream of any settlement and agricultural activity (49°14′N; 8°03′E), while *B. rhodani* nymphs were obtained approximately 2 km downstream, where agricultural influence is negligible, however, the reduced canopy ensures the provision of food in form of algae for *Baetis* nymphs resulting in higher abundances. Animals were collected 24 h prior to the start of each experiment by kick sampling. Subsequently, gammarids and mayfly nymphs were kept separately in aerated stream water from the Hainbach at 20 ± 1 °C. Meanwhile *G. fossarum* were fed with conditioned leaf material and *B. rhodani* nymphs received algae-covered stones from the sampling site.

Specimens of *G. fossarum* were visually checked for acanthocephalan parasites prior to the start of each experiment, since parasites may affect gammarids' predatory behavior (Dick et al., 2010). Moreover, egg-carrying female gammarids were excluded due to their anticipated higher sensitivity (McCahon and Pascoe, 1988). Since, differences in the sensitivity between males and non-egg-carrying females are unlikely (Malbouisson et al., 1995), both sexes of gammarids were used in the present study. Only *Baetis* nymphs and adult gammarids (judged by body length) of approximately 0.7–1.0 cm and 0.6–0.8 cm (Pöckl, 1992), respectively, were randomly allocated to the bioassay, which reduced between treatment variability related to gender and body size of the test organisms.

2.4. Bioassay

For each of the four experiments, 13 (1.00 μg/L) to 17 (0.50, 0.75 and 4.00 μg/L) replicates were set up for both the thiacloprid treatment and the corresponding control. Ten *B. rhodani* nymphs and five *G. fossarum* specimens were placed together with five re-soaked preweighed leaf discs in a 900-mL crystallizing dish containing 500 mL stream water with the respective thiacloprid concentration, namely 0.00, 0.50, 0.75, 1.00 or 4.00 μg active ingredient/L. Each test vessel further contained one pebble (diameter: ∼4 cm), which served as substrate. As thiacloprid might partly adsorb to the offered leaf discs, *Gammarus* may experience both aqueous and dietary exposure. However, the contribution of each of the exposure pathways to the effect on the test organisms was not the scope of the present study.

Each experiment was accompanied by five replicates without gammarids and thiacloprid but with *Baetis* nymphs accounting for *Baetis*-mediated, abiotic and microbial leaf mass loss. Test vessels were placed randomized in a climate chamber at 20 ± 1 °C in complete darkness and were covered with petri dishes (headspace between water and petri dish ∼ 3 cm) to prevent evaporation of test medium and the loss of emerging mayflies.

Table 2
Nominal and measured (mean ± 95% CI; $n = 3$) thiacloprid concentrations. Limit of quantification (LOQ = 0.18 μg/L).

Nominal concentration (μg/L)	Measured concentration (μg/L)
0.00	<LOQ
0.50	0.40 ± 0.22
0.75	0.67 ± 0.19
1.00	1.19 ± 0.04
4.00	3.50 ± 0.50

Every 12 h, the numbers of alive, dead, emerged and consumed *B. rhodani* nymphs were recorded. Mayfly nymphs were considered as consumed if less than 50% of their bodies remained. Consumed mayflies as well as leaf discs were not replaced to avoid any implication on the predator–prey-interaction that may have covered the toxic effect of thiacloprid. Gammarids were also checked every 12 h for mortality. Dead *G. fossarum* were immediately removed from the test vessels to prevent cannibalism. Gammarids were considered dead if no response was observable after several gentle touches with the tip of a glass pipette. The study duration was set at 96 h, which is justified by a nearly linearly increasing predation over the whole study duration (Fig. S1) indicating that gammarids never experienced a limitation regarding the availability of prey specimen given the study design used. At the end of the experiments, all individuals of *G. fossarum*, remaining leaf discs and any visible leaf tissue shredded off were removed from the test vessels with a featherweight forceps, dried separately at 60 °C to constant weight and subsequently weighed to the nearest 0.01 mg.

2.5. Calculations and statistics

The predation (P) of *G. fossarum* on *B. rhodani* was expressed as percentage of consumed nymphs at each point of time and calculated as:

$$P_t = \frac{z_t}{(N - E_t)}*100 \tag{1}$$

where Z_t is the number of consumed nymphs at time t, N the initial number of nymphs per treatment and E_t the number of emerged nymphs until time t.

Leaf consumption of *G. fossarum* (C) was expressed as mg consumed leaf material per mg gammarid biomass and day and calculated as follows (Naylor et al., 1989):

$$C = \frac{L_b * k - L_e}{g * T} \tag{2}$$

where L_b is the initial dry weight of the leaf discs, L_e the final dry weight of the leaf discs, g the dry weight of gammarids per replicate and T the feeding time in days. The leaf change correction factor k was given by

$$k = \frac{\sum\left(\frac{L_{oe} - L_{oe}}{L_{ob}}\right)}{n} \tag{3}$$

where L_{ob} is the initial dry weight of the leaf discs, L_{oe} the final dry weight of the leaf discs – both measured in replicates without gammarids present – and n the number of replicates.

Significance testing of this study was based on unpaired two-sided 95% confidence intervals (CIs), because of existing shortcomings associated with the conventional application of null hypothesis significance testing (e.g. Nakagawa and Cuthill, 2007; Newman, 2008). Depending on the data, either means or medians, were analyzed using the corresponding methods (Altman et al., 2000). If CIs of differences between treatments did not include zero, the test outcome was judged as significant. To obtain approximate p-values, 95%, 99% and 99.9% CIs were calculated corresponding with p-values of 0.05, 0.01 and 0.001, respectively. The term significant(ly) is exclusively used with reference to statistical significance throughout the present study. For statistics and figures, R version 2.11.1 was used (R Development Core Team, 2010).

3. Results & discussion

Thiacloprid exposure clearly affected the interaction between the predator *G. fossarum* and its prey *B. rhodani* at concentrations below those reported to occur in the field e.g. by Süß et al. (2006). The predation expressed as percent of *Baetis* nymphs consumed by *Gammarus* was significantly increased at the three lowest thiacloprid concentrations assessed in the present study after 96 h of exposure (0.50 μg/L: difference of means: 13.9%; 95% CI 1.6 to 26.1; $p < 0.05$; $n = 17$; 0.75 μg/L: difference of medians: 20.0%; 95% CI 10.0 to 30.0; $p < 0.001$; $n = 17$; 1.00 μg/L: difference of means: 36.8%; 95% CI 22.1 to 51.4; $p < 0.001$; $n = 13$; Fig. 1; Fig. S1). Although *Gammarus* may have experienced reduced prey densities (Hollings, 1966) during the 96-h test duration, its functional response seems to be unaffected as the predation increased nearly linear over time (Fig. S1). The shifts in predation by *Gammarus* went along with reduced leaf consumption. At 0.75 and 1.00 μg thiacloprid/L this endpoint was with 15.7% (difference of means: 0.06 mg/mg gammarid/d; 95% CI: >0.0 to 0.1; $p < 0.05$; $n = 16$) and 41.4% (difference of means: 0.13 mg/mg gammarid/d; 95% CI: 0.1 to 0.2; $p < 0.001$; $n = 11$), respectively, significantly reduced if compared to the respective control (Fig. 2). As *G. fossarum* is considered a key-species in the leaf litter breakdown (Dangles et al., 2004), such a decrease in leaf consumption may translate directly into an impairment of this ecosystem function (Maltby et al., 2002). Moreover, gammarids usually prefer food with the highest nutritional value (Bundschuh et al., 2011a). Since thiacloprid increased the predation by *Gammarus* – most likely by impairing the predator avoidance behavior of *B. rhodani* (cp. Schulz and Dabrowski, 2001;

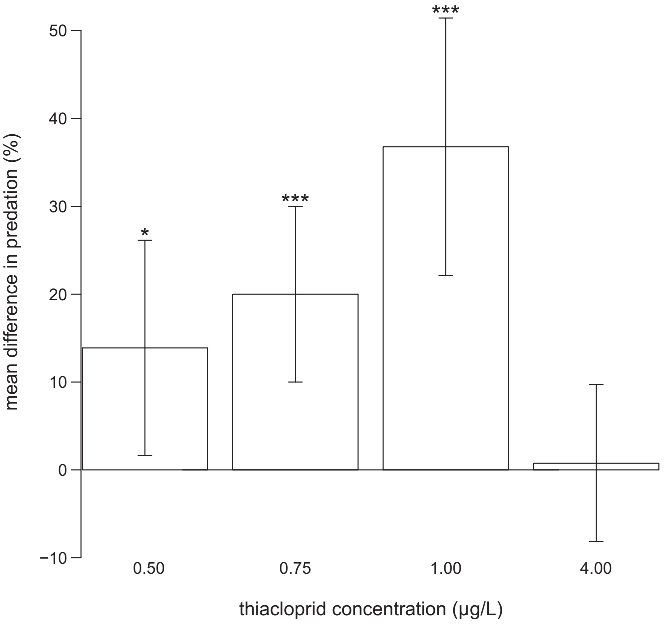

Fig. 1. Mean differences in predation (with 95% CIs) by *G. fossarum* on *B. rhodani* nymphs between each thiacloprid treatment and the corresponding control following 96 h of exposure. Asterisk denotes a significant difference compared to the corresponding control, $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

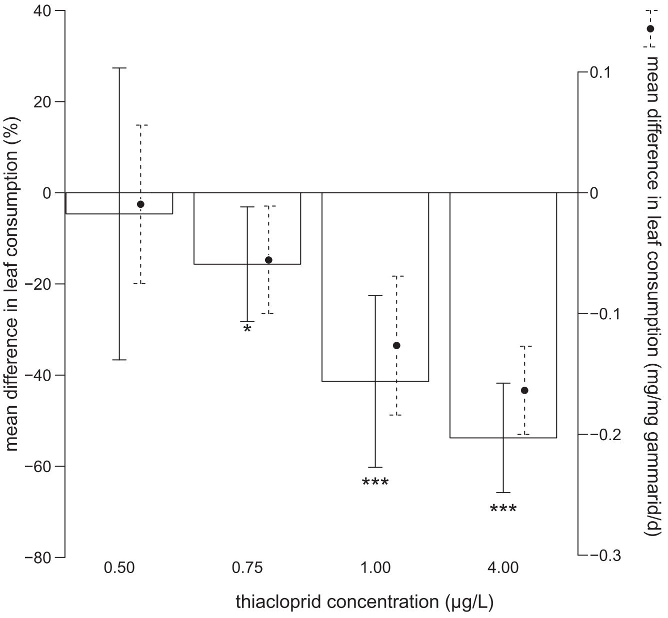

Fig. 2. Mean relative (barplot) and absolute (●) differences in leaf consumption (with 95% CIs) of *G. fossarum* between each thiacloprid treatment and the corresponding control following 96 h of exposure. Asterisk denotes a significant difference compared to the corresponding control, $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Weis et al., 2001) – the observed reduction in leaf consumption seems to be a result of an indirect mechanism. This assumption is further supported by a leaf-feeding trial by Feckler et al. (submitted for publication) conducted with *G. fossarum* but in absence of *B. rhodani*, while exposed to concentrations from 0.50 to 50.00 μg thiacloprid/L. The authors reported no adverse effects in gammarids' leaf consumption at thiacloprid concentrations up to 1.00 μg/L following seven days of exposure. This makes any direct ecotoxicological effect of thiacloprid on *G. fossarum* at these concentrations unlikely during the present study.

Although all *Baetis* nymphs died within 24 h (data not shown) at the 4.00 μg thiacloprid/L-experiment the predation by *G. fossarum* deviated only marginally from the corresponding control during the study duration of 96 h (difference of means: 0.8%; 95% CI 0.0 to 9.7; $p > 0.05$; $n = 17$; Fig. 1 & Fig. S1). At the same time, gammarids' leaf consumption was significantly reduced by 53.8% (difference of means: 0.16 mg/mg gammarid/d; 95% CI: 0.1 to 0.2; $p < 0.001$; $n = 17$; Fig. 2). As gammarids prefer the most nutritious food available (Arsuffi and Suberkropp, 1989; Bundschuh et al., 2011a) and even fed on dead animal matter (Gergs and Rothhaupt, 2008), it would have been expected that *Baetis* nymphs are consumed in higher quantities. However, Feckler et al. (submitted for publication) reported at 5.00 μg thiacloprid/L a significantly reduced leaf consumption of *G. fossarum*. Hence, it may be hypothesized that direct ecotoxicological effects of thiacloprid on gammarids may have outcompeted the potential beneficial effects of an altered prey availability due to affected *B. rhodani* nymphs.

The thiacloprid-mediated shifts in the predation and leaf consumption of *G. fossarum* either with increasing insecticide concentration or compared to the respective control, seemed to affect also the dry weight gained by *Gammarus* during the test period. Following the 96-h exposure to 1.00 μg thiacloprid/L, gammarids' dry weight was significantly increased by approximately 15% (difference of means: 2.58 mg; 95% CI: 0.1 to 5.0; $p < 0.05$; $n = 14$) compared to the corresponding control (Fig. 3). This elevated growth of *G. fossarum* may be attributed to the increased consumption of animal matter (Fig. 1 & Fig. S1), which is of high nutritional value, while leaf material (lower nutritional value; cp. Gergs and Rothhaupt, 2008) was less consumed (Fig. 2). In contrast, at 4.00 μg thiacloprid/L the *Gammarus* dry weight was with approximately 20% (difference of means: of 2.57 mg; 95% CI: 1.4 to 3.8; $p < 0.001$; $n = 17$) significantly decreased compared to the corresponding control (Fig. 3). This reduced physiological fitness in terms of growth may be explained by reduced leaf consumption of approximately 55% (Fig. 2), which was not compensated for through an increased predation upon mayfly nymphs (Fig. 1 & Fig. S1). Moreover, a generally increased energy demand under toxic stress (cp. Maltby, 1999) may also be a relevant factor for the reduction in the gammarids dry weight at the highest thiacloprid concentration as observed in the present study.

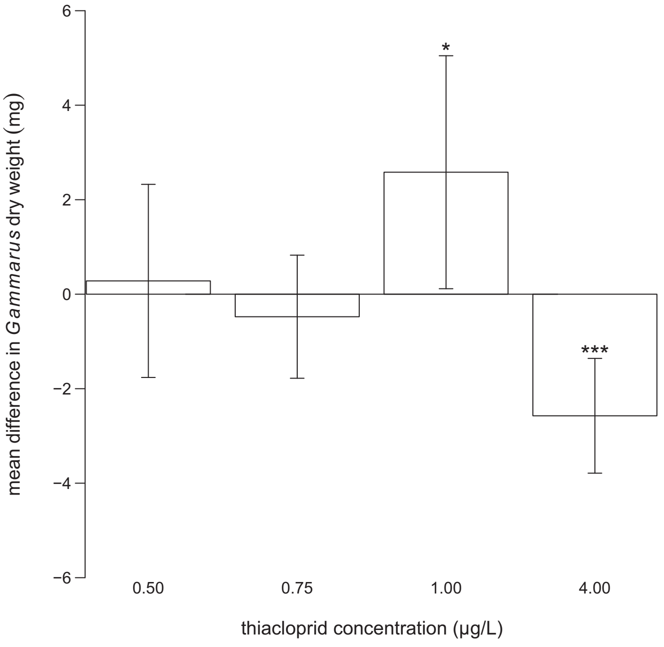

Fig. 3. Mean differences in dry weight (with 95% CIs) of *G. fossarum* between each thiacloprid treatment and the corresponding control following 96 h of exposure. Asterisk denotes a significant difference compared to the corresponding control, $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

These results show that the predation by *G. fossarum* upon mayflies increases with increasing thiacloprid concentration, up to a concentration of 1.00 μg/L, which might thereby have at least short-term implications for mayfly populations during insecticide peak exposure events. Moreover, it may be hypothesized that in response to sublethal toxicant exposure (Riddell et al., 2005; Brooks et al., 2009) or substantially reduced mayfly abundances (Hollings, 1966), *Gammarus* may show a shift toward other species for predation. Therefore, these results might also be valid for interactions of gammarids with other stream insect taxa (e.g. Trichoptera, Plecopteraa and Diptera), which *Gammarus* preys upon as well (see Felten et al., 2008). By transferring these findings to the field situation, it is conceivable that gammarid populations may benefit from contamination below the threshold concentration of 1.00 μg thiacloprid/L obtained in the present study by substantially increasing predation on mayfly nymphs. This can be hypothesized since higher proportions of animal matter in the diet of *Gammarus* may result, at least temporarily, in enhanced growth and reproduction (cp. Maltby, 1994). Hence, an indirectly increased predation by gammarids due to exposure to neurotoxic substances (e.g. neonicotinoids) may affect the community composition (cp. Wooster, 1994) within aquatic ecosystems. Besides this thiacloprid induced enhanced predation by gammarids, elevated neonicotinoid field concentrations as reported by Süß et al. (2006) may impair the survival of insect nymphs directly. Additionally, sublethal responses e.g. impaired nymph development as reported by Alexander et al. (2008) during a field-mesocosm experiment might occur at markedly lower neonicotinoid concentrations and may therefore, scale the emergence of insects further down (Pestana et al., 2009b). This finally may limit the energy transfer from aquatic to terrestrial ecosystems (e.g. Polis et al., 1997; Knight et al., 2005) and consequently terrestrial insectivore predators (e.g. spiders, birds and bats) that partly rely on aquatic prey are potentially affected (Nakano and Murakami, 2001; Paetzold et al., 2005; Fukui et al., 2006; Stahlschmidt et al., in press).

Despite the vast number of studies dealing with toxic effects on predator–prey-interactions (e.g. Junges et al., 2010), their implications at the population and community level as well as the underlying processes influencing ecosystem functions e.g. leaf litter breakdown remain largely unclear (see also Bundschuh et al., in press). Our findings suggest that neonicotinoid insecticides impair the food selection of gammarids as well as reduce invertebrate-mediated leaf consumption. The latter may translate into alterations in the ecosystem function of leaf litter breakdown, at least temporarily (cp. Pestana et al., 2009b). Consequently, the amount and quality of feces produced by aquatic animals, which depends on their feeding-strategy and assimilation efficiency (Wotton and Malmqvist, 2001) may affect feces-feeding species of downstream communities.

4. Conclusions

Although neonicotinoid insecticides like thiacloprid are highly toxic for aquatic insects, their implications for aquatic food webs and finally ecosystem functions are hardly investigated (cp. Beketov et al., 2008; Kreutzweiser et al., 2007, 2009). The present study clearly displayed that thiacloprid increases predation by *G. fossarum* while reducing its leaf consumption, which hence might alter species interactions at environmentally relevant concentrations. Therefore, macroinvertebrate diversity in the field may in turn be adversely affected. However, in order to identify and understand relevant processes causing such shifts at the level of ecosystems, microcosm experiments – as applied during the present study – that reduced the complexity of interactions may be a suitable tool.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2012.03.024.

References

Alexander, A.C., Heard, K.S., Culp, J.M., 2008. Emergent body size of mayfly survivors. Freshw. Biol. 53, 171–180.

Altman, D.A., Machin, D., Bryant, T., Gardner, M.J. (Eds.), 2000. Statistics with Confidence: Confidence Intervals and Statistical Guidelines, 2nd ed. BMJ, London.

Arsuffi, T.L., Suberkropp, K., 1989. Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. Oecologia 79, 30–37.

Bärlocher, F., 1985. The role of fungi in the nutrition of stream invertebrates. Bot. J. Linn. Soc. 91, 83–94.

Bauernfeind, E., Moog, O., 2000. Mayflies (Insecta: Ephemeroptera) and the assessment of ecological integrity: a methodological approach. Hydrobiologia 422/423, 71–83.

Beketov, M.A., Liess, M., 2008a. Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. Environ. Toxicol. Chem. 27, 461–470.

Beketov, M.A., Liess, M., 2008b. Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. Freshw. Biol. 55, 247–253.

Beketov, M.A., Schäfer, R.B., Marwitz, A., Paschke, A., Liess, M., 2008. Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: effect concentrations and recovery dynamics. Sci. Total Environ. 405, 96–108.

Brittain, J.E., 1982. Biology of mayflies. Annu. Rev. Entomol. 27, 119–147.

Brooks, A.C., Gaskell, P.N., Maltby, L., 2009. Sublethal effects and predator-prey interactions: implications for ecological risk assessment. Environ. Toxicol. Chem. 28, 2449–2457.

Bundschuh, M., Zubrod, J.P., Kosol, S., Maltby, L., Stang, C., Duester, L., Schulz, R., 2011a. Fungal composition on leaves explains pollutant-mediated indirect effects on amphipod feeding. Aquat. Toxicol. 104, 32–37.

Bundschuh, M., Zubrod, J.P., Schulz, R., 2011b. The functional and physiological status of *Gammarus fossarum* (Crustacea; Amphipoda) exposed to secondary treated wastewater. Environ. Pollut. 159, 244–249.

Bundschuh, M., Appeltauer, A., Dabrunz, A., Schulz, R., in press. Combined effect of invertebrate predation and sublethal pesticide exposure on the behavior and survival of *Asellus aquaticus* (Crustacea; Isopoda). Arch. Environ. Contam. Toxicol.

Carter, A., 2000. How pesticides get into water – and proposed reduction measures. Pestic. Outlook 11, 149–156.

Dang, C.K., Chauvet, E., Gessner, M.O., 2005. Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. Ecol. Lett. 8, 1129–1137.

Dangles, O.J., Gessner, M.O., Guérold, F.A., Chauvet, E., 2004. Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. J. Appl. Ecol. 41, 365–378.

Delong, M.D., Summers, R.B., Thorp, J.H., 1993. Influence of food type on the growth of a riverine amphipod, *Gammarus fasciatus*. Can. J. Fish Aquat. Sci. 50, 1891–1896.

Dewey, S.L., 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology 67, 148–162.

Dick, J.T.A., Armstrong, M., Clarke, H.C., Farnsworth, K.D., Hatcher, M.J., Ennis, M., Kelly, A., Dunn, A.M., 2010. Parasitism may enhance rather than reduce the predatory impact of an invader. Biol. Lett. 6, 636–638.

Feckler, A., Thielsch, A., Schwenk, K., Schulz, R., Bundschuh, M., submitted for publication. Morphologically similar but genetically different: are cryptic lineages comparable in their sensitivity towards chemical stress?

Felten, V., Tixier, G.F., De Crespin De Billy, V., Dangles, O.J., 2008. Quantification of diet variability in a stream amphipod: implications for ecosystem functioning. Fundam. Appl. Limnol. 170, 303–313.

FOOTPRINT, 2011. The FOOTPRINT Pesticide Properties Database. In: Database Collated by the University of Hertfordshire as Part of the EU-funded FOOTPRINT Project (FP6-SSP-022704). www.eu-footprint.org/ppdb.html (accessed 23.08.11).

Fukui, D.A.I., Murakami, M., Nakano, S., Aoi, T., 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. J. Anim. Ecol. 75, 1252–1258.

Gergs, R., Rothhaupt, K., 2008. Feeding rates, assimilation efficiencies and growth of two amphipod species on biodeposited material from zebra mussels. Freshw. Biol. 53, 2494–2503.

Habdija, I., 1986. Einfluß der organischen Belastung auf die biozönotische Struktur des Makrozoobenthos in lithorheophilen, psammorheophilen und pelorheophilen Zönosen der Gebirgsbäche. Acta Hydrochim. Hydrobiol. 14, 241–254.

Hollings, C.S., 1966. The functional response of invertebrate predators to prey density. Mem. Entomol. Soc. Can. 98, 1–86.

Jackson, J.K., Fisher, S.G., 1986. Secondary production, emergence, and export of aquatic insects of a sonoran desert stream. Ecology 67, 629–638.

Jeschke, P., Moriya, K., Lantzsch, R., Seifert, H., Lindner, W., Jelich, K., Gohrt, A., Beck, M.E., Etzel, W., 2001. Thiacloprid (Bay YRC 2894) – a new member of the chloronicotinyl insecticide (CNI) family. Pflanzenschutz-Nachrichten Bayer 54, 147–160.

Junges, C.M., Lajmanovich, R.C., Peltzer, P.M., Attademo, A.M., Bass, A., 2010. Predator–prey interactions between *Synbranchus marmoratus* (Teleostei: Synbranchidae) and *Hypsiboas pulchellus* tadpoles (Amphibia: Hylidae): importance of lateral line in nocturnal predation and effects of fenitrothion exposure. Chemosphere 81, 1233–1238.

Kelly, D.W., Dick, J.T.A., Montgomery, W.I., 2002a. Predation on mayfly nymph, *Baetis rhodani*, by native and introduced *Gammarus*: direct effects and the facilitation of predation by salmonids. Freshw. Biol. 47, 1257–1268.

Kelly, D.W., Dick, J.T.A., Montgomery, W.I., 2002b. The functional role of *Gammarus* (Crustacea, Amphipoda): shredders, predators, or both? Hydrobiologia 485, 199–203.

Knight, T.M., McCoy, M.W., Chase, J.M., McCoy, K.A., Holt, R.D., 2005. Trophic cascades across ecosystems. Nature 437, 880–883.

Kreutzweiser, D.P., Good, K., Chartrand, D., Scarr, T.A., Thompson, D.G., 2007. Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. Ecotoxicol. Environ. Saf. 68, 315–325.

Kreutzweiser, D.P., Thompson, D.G., Scarr, T.A., 2009. Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. Ecotoxicol Environ. Saf. 72, 1053–1057.

Ladewig, V., Jungmann, D., Köhler, H.R., Schirling, M., Triebskorn, R., Nagel, R., 2006. Population structure and dynamics of *Gammarus fossarum* (Amphipoda) upstream and downstream from effluents of sewage treatment plants. Arch. Environ. Contam. Toxicol. 50, 370–383.

MacNeil, C., Dick, J.T.A., Elwood, R.W., 1999. The dynamics of predation on *Gammarus* spp. (Crustacea: Amphipoda). Biol. Rev. 74, 375–395.

Malbouisson, J.F.C., Young, T.W.K., Bark, A.W., 1995. Use of feeding rate and repairing of precopulatory *Gammarus pulex* to assess toxicity of gamma-hexachlorocyclohexane (lindane). Chemosphere 30, 1573–1583.

Maltby, L., Clayton, S.A., Wood, R.M., McLoughlin, N., 2002. Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality robustness, responsiveness, and relevance. Environ. Toxicol. Chem. 21, 361–368.

Maltby, L., 1994. Stress, shredders and streams: using *Gammarus* energetics to assess water quality. In: Sutcliffe, D.W. (Ed.), Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking Levels of Organisation, pp. 98–110.

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

McCahon, C., Pascoe, D., 1988. Increased sensitivity to cadmium of the freshwater amphipod *Gammarus pulex* (L.) during the reproductive period. Aquat. Toxicol. 13, 183–193.

Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol. Rev. 82, 591–605.

Nakano, S., Murakami, M., 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. PNAS 98, 166–170.

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

Newman, M.C., 2008. "What exactly are you inferring?" A closer look at hypothesis testing. Environ. Toxicol. Chem. 27, 1013–1019.

Paetzold, A., Schubert, C., Tockner, K., 2005. Aquatic terrestrial linkages along a braided-river: riparian arthropods feeding on aquatic insects. Ecosystems 8, 748–759.

Peckarsky, B.L., Cowan, C.A., Penton, M.A., Anderson, C., 1993. Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74, 1836–1846.

Peckarsky, B.L., Taylor, B.W., McIntosh, A.R., McPeek, M.A., Lytle, D.A., 2001. Variation in mayfly size at metamorphosis as a developmental response to risk of predation. Ecology 82, 740–757.

Pestana, J.L.T., Loureiro, S., Baird, D.J., Soares, A.M.V.M., 2009a. 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, J.L.T., Alexander, A.C., Culp, J.M., Baird, D.J., Cessna, A.J., Soares, A.M.V.M., 2009b. Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. Environ. Pollut. 157, 2328–2334.

Piscart, C., Genoel, R., Doledec, S., Chauvet, E., Marmonier, P., 2009. Effects of intense agricultural practices on heterotrophic processes in streams. Environ. Pollut. 157, 1011–1018.

Piscart, C., Navel, S., Maazouzi, C., Montuelle, B., Cornut, J., Mermillod-Blondin, F., des Chatelliers, M.C., Simon, L., Marmonier, P., 2011. Leaf litter recycling in benthic and hyporheic layers in agricultural streams with different types of land use. Sci. Total Environ. 409, 4373–4380.

Pöckl, M., 1992. Effects of temperature, age and body size on moulting and growth in the freshwater amphipods *Gammarus fossarum* and *G. roeseli*. Freshw. Biol. 27, 211–225.

Pöckl, M., 1993. Reproductive potential and lifetime potential fecundity of the freshwater amphipods *Gammarus fossarum* and *G. roeseli* in Austrian streams and rivers. Freschw. Biol. 30, 73–91.

Polis, G.A., Anderson, W.B., Holt, R.D., 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. 28, 289–316.

R Development Core Team, 2010. R: a language and environment for statistical computing, 2nd edn. R Foundation for Statistical Computing. www.R-project.org.

Riddell, D.J., Culp, J.M., Baird, D.J., 2005. Sublethal effects of cadmium on prey choice and capture efficiency in juvenile brook trout (*Salvelinus fontinalis*). Environ. Toxicol. Chem. 24, 1751–1758.

Schmuck, R., 2001. Ecotoxicological profile of the insecticide thiacloprid. Pflanzenschutz-Nachrichten Bayer 54, 161–184.

Schulz, R., Dabrowski, J.M., 2001. Combined effects of predatory fish and sublethal pesticide contamination on the behavior and mortality of mayfly nymphs. Environ. Toxicol. Chem. 20, 2537–2543.

Schulz, R., Liess, M., 2001. Runoff simulation with particle-bound fenvalerate in multispecies stream microcosms: importance of biological interactions. Environ. Toxicol. Chem. 20, 757–762.

Schulz, R., 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution. J. Environ. Qual. 33, 419–448.

Song, M.Y., Stark, J.D., Brown, J.J., 1997. Comparative toxicology of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. Environ. Toxicol. Chem. 16, 2494–2500.

Stahlschmidt, P., Pätzold, A., Ressl, L., Schulz, R., Brühl, C., in press. Constructed wetlands support bats in agricultural landscapes. Basic Appl. Ecol.

Stenersen, J., 2004. Chemical Pesticides Mode of Action and Toxicology. CRC Press, Hoboken.

Süß, A., Bischoff, G., Mueller, A.C.W., Buhr, L., 2006. Chemisch-biologisches Monitoring zu Pflanzenschutzmittelbelastungen und Lebensgemeinschaften in Gräben des Alten Landes. Nachrichtenbl. Deut. Pflanzenschutzd 58, 28–42.

Tennekes, H.A., 2010. The Systemic Insecticides: A Disaster in the Making. Northern Bee Books, West Yorkshire.

Weis, J.S., Smith, G., Zhou, T., Santiago-Bass, C., Weis, P., 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience 51, 209–217.

Wooster, D.E., 1994. Predator impacts on stream benthic prey. Oecologia 99, 7–15.

Wotton, R.S., Malmqvist, B., 2001. Feces in aquatic ecosystems. BioScience 51, 537–544.