



# Predator cues magnify effects of the pesticide endosulfan in water bugs in a multi-species test in outdoor containers



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## ABSTRACT

Pesticides have become major stressors in many aquatic communities. Laboratory studies suggest their impact may be further magnified in the presence of cues from predators. Despite their importance for ecological risk assessment, synergisms between pesticides and predator cues have not been confirmed under semi-natural outdoor conditions. We evaluated how the presence of predator cues and the presence of a non-corixid community affect the pesticide sensitivity of five water bug (Corixidae) species in an outdoor, multi-species container experiment. The experiment employed a full factorial design with two pesticide treatments, two predator cue treatments and two (non-corixid) community treatments (absence versus presence of *Cloeon dipterum* mayfly larvae, *Ischnura elegans* damselfly larvae and *Physa acuta* snails). The pesticide treatment negatively affected survival in *Cymatia coleoptrata*, and to a lesser extent, *Sigara lateralis*, but not in the other three Corixidae species (*Hesperocorixa linnaei*, *Sigara iactans* and *Sigara striata*). The addition of pesticides did not significantly affect body mass in the latter four species, unless combined with predator cues. To our knowledge this is the first report of this synergism under semi-natural, outdoor conditions. Neither lethal nor sublethal pesticide effects in the Corixidae depended on the community context, yet the presence of the non-corixid community when combined with predator cues reduced survival and body mass. Our results suggest that the here documented synergism between pesticides and predator cues may occur in nature.

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## 1. Introduction

Freshwater habitats worldwide are facing a biodiversity crisis due to increasing human impact (Dudgeon et al., 2006; Voeroesmarty et al., 2010). Moreover, aquatic organisms also face natural stressors such as suboptimal abiotic conditions and natural enemies that potentially magnify the effects of anthropogenic stressors (Holmstrup et al., 2010; Laskowski et al., 2010). There is increasing concern that the prevalence and magnitude of interactions among stressors may result in accelerated biodiversity loss (Sala et al., 2000), yet such synergisms remain one of the largest uncertainties when predicting future ecological change (Darling and Côté, 2008; Lindenmayer et al., 2010). Therefore, the study of interactions between anthropogenic and natural stressors is crucial

to our understanding and ability to project how natural aquatic communities will change with increasing human impact.

Anthropogenic stressors that have received much attention in this context are pesticides. These pollutants are increasingly used worldwide (Tilman et al., 2001) and present a major threat to many natural aquatic ecosystems (Butchart et al., 2010; Relyea and Hoverman, 2006; Schwarzenbach et al., 2006). A natural stressor that is especially important in aquatic ecosystems is the stress imposed by predator cues (Preisser et al., 2005). The non-consumptive effects imposed by predators can be as important or even more important for prey population dynamics than the consumptive effects (Preisser et al., 2005). There is a growing literature indicating that the effects of pesticide exposure and predator cues may not always add up, and that predator cues may magnify the impact of pesticide stress (e.g. Campero et al., 2007; Relyea, 2004, 2003; Relyea and Mills, 2001). Such synergistic interactions between pesticides and predator cues are, however, not always detected (see e.g., Pestana et al., 2009; Qin et al., 2011), and both stressors can sometimes also interact antagonistically (Relyea, 2012). Currently, it is poorly understood under which conditions the presence of synergisms is most likely. Moreover, nearly all studies tested for the presence of these synergisms using laboratory experiments whereas we need to know whether they also

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occur under more natural conditions (Relyea, 2012; Relyea and Hoverman, 2006). Of the three studies that tested for the presence of a synergism between pesticides and predator cues under semi-natural outdoor conditions, none could demonstrate its presence (Relyea, 2012, 2006; Rohr and Crumrine, 2005).

The increasing number of studies showing interactions between pesticides and natural stressors identified the need for a community-level approach when evaluating the impact of pesticides (Relyea et al., 2005; Relyea and Hoverman, 2006; Rohr et al., 2006). Beside predation, other biotic interactions within the community also may play a role, yet have been much less considered in ecotoxicology (Clements and Newman, 2002, but see e.g. Buck et al., 2012; Jones et al., 2011; Rohr and Crumrine, 2005). For example, competitive interactions and food shortage may directly affect the response to a pesticide (Boone and Semlitsch, 2002; Rohr and Crumrine, 2005), but also may modulate the expression of interactions between pesticides and predator cues. Yet, only very few studies jointly manipulated pesticide stress, predator cues and interactions with a community (but see Rohr and Crumrine, 2005). Documenting interactions with pesticide stress and to what extent they themselves depend on other stressors is an important step to arrive at a predictive community ecotoxicology (Clements and Rohr, 2009; Rohr et al., 2006).

In the current study, we investigated the combined effects of pesticide stress, predator cues and the presence of a non-corixid community on five Corixidae (Hemiptera) species. By applying a full factorial design in an outdoor container experiment, we obtained a gradient from a more artificial (only Corixidae) to a more natural setting (including predator cues and a non-corixid community). As predator cues, pesticides and competition may be important for prey population dynamics (Preisser et al., 2005), we quantified effects on survival and per capita dry mass, key traits linked to fitness. Our main objective was to test the independent and interacting effects of pesticide effects, predator cues and a non-corixid community in a full factorial design.

## 2. Materials and methods

### 2.1. Corixidae species

Corixidae are common inhabitants of all types of lentic waters, and feed on small invertebrates, detritus and algae (Savage, 1989). The species of Corixidae used in this study were *Cymatia coleoptrata* (Fabricius, 1777), *Hesperocorixa linnaei* (Fieber, 1848), *Sigara iactans* (Jansson, 1983), *Sigara lateralis* (Leach, 1817) and *Sigara striata* (Linnaeus, 1758), all of which commonly co-occur in ponds in Belgium. Corixidae were caught in August 2008 from ponds located in Flanders that have no known pesticide application history, and that were surrounded by natural land use.

### 2.2. Experimental setup

We set up an outdoor container experiment to evaluate how the presence of predator cues and the presence of a non-corixid community affect the pesticide sensitivity of the five water bug (Corixidae) species under semi-natural conditions. The experiment employed a full factorial design with two pesticide treatments  $\times$  two predator cue treatments  $\times$  two (non-corixid) community treatments. Each treatment combination contained all five Corixidae species, and was replicated in three containers (total of 24 containers).

For the pesticide treatment, we chose to use the pesticide endosulfan, which is a neurotoxic chlorinated cyclodiene insecticide that stimulates the central nervous system through the GABA-receptor (Stenersen, 2004). We chose this pesticide because it is a global

insecticide (Weber et al., 2010), shown to interact under laboratory conditions with predator cues in aquatic insects (Campero et al., 2007) including Corixidae (Trekels et al., 2012). Some studies also showed increased mortality by predation in the presence of endosulfan (Broomhall, 2002; Carlson et al., 1998). In Belgium, concentrations of up to 54.5  $\mu\text{g/L}$  have been measured recently (VMM Flemish Environment Agency, 2006, unpublished data), but elsewhere peak concentrations might be as high as 700  $\mu\text{g/L}$  (Ernst et al., 1991). Before endosulfan was banned in Belgium in 2007, concentrations exceeded the water quality standards of 10  $\text{ng/L}$  in ca. 30% (2005–2006) of all sample locations of the Flemish government, including several observations of concentrations over 10  $\mu\text{g/L}$  annually (Van Steertegeem, 2012). Five years later, and after endosulfan had been banned, concentrations of this persistent organic pollutant and its metabolites still exceeded these limits in 10% of the sample locations (Van Steertegeem, 2012). According to the species sensitivity distribution of endosulfan, ca. 50% of all freshwater species would be affected at the chosen concentration of 8  $\mu\text{g/L}$  (Wan et al., 2005). The toxicity values of some often used aquatic test organisms are the following: LC50 48 h = 1.0  $\mu\text{g/L}$  for the salmonid fish *Oncorhynchus kisutch*, LC50 48 h = 840  $\mu\text{g/L}$  for the cladoceran *Daphnia magna* and LC50 96 h = 5.7  $\mu\text{g/L}$  for the freshwater amphipod *Hyallorella azteca* (Wan et al., 2005).

We used endosulfan  $\alpha:\beta$  1:2 from Sigma Aldrich® (Pestanal class, purity  $\geq 98.9$ ). At the start of the exposure period, endosulfan from a 1000  $\text{mg/L}$  stock solution (solvent: acetone) was added once to install the nominal pesticide concentrations of 8  $\mu\text{g/L}$ . This mimicked a realistic pulse pesticide application. This nominal concentration (8  $\mu\text{g/L}$ ) was chosen based on the results of previous experiments where it had caused species-specific lethal and sub-lethal (i.e. growth rate) effects (Trekels et al., 2012, 2011). All containers, including the pesticide-free ones, received the same amount (800  $\mu\text{L}$ ) of acetone. Water samples for pesticide analyses were taken by pooling samples across all containers of the pesticide treatment at the start of the experiment and after ten days. Concentrations of endosulfan ( $\alpha$ -,  $\beta$ - and -sulphate) were analyzed by gas chromatography with electron capture detection (column: cp-Sil 8CB 50 m 0.39  $\mu\text{m}$ ; 1.38 bar; carrier: He; injection: 250 °C; detection: 275 °C) after liquid extraction in dichloromethane. The initial endosulfan concentration was 6.68  $\mu\text{g/L}$  in the treatment with pesticide presence. In line with the rapid dissipation of endosulfan (Stenersen, 2004), the endosulfan level was reduced to 0.078  $\mu\text{g/L}$  after ten days. This residual fraction is comparable with previous container experiments (Trekels et al., 2011).

The two predator cue treatments were absence and presence of a caged pumpkinseed sunfish, *Lepomis gibbosus* (Linnaeus, 1758). This species has become widespread in Flanders after the first introduction in 1885 (Verreycken et al., 2007), and is a major predator of Corixidae (F. Van de Meutter, unpublished data). All fish were collected in a pesticide-free lake in a nature reserve in Genk (Belgium). In the treatment with predator cues, one pumpkinseed sunfish (standard length ca. 6 cm) was placed in a transparent cylindrical cage (diameter: 20 cm) with two 5 cm  $\times$  5 cm holes on opposite sides covered by 200  $\mu\text{m}$  mesh to provide both visual and chemical predator cues while excluding consumptive predation. Fish were fed 20 frozen chironomid larvae every two days. The containers of the treatment without predator cues had similar but empty cages. If a fish died, it was replaced within 24 h.

For the community treatment, a set of non-corixid macroinvertebrates was added to the containers. We used mayfly larvae (*Cloeon dipterum* Linnaeus, 1761), damselfly larvae (*Ischnura elegans* Vander Linden, 1820), and snails (*Physa acuta* Draparnaud, 1805). This simplified community was composed of the three most abundant species that commonly co-occurred in the ponds sampled (H. Trekels and F. Van de Meutter, unpublished data), and that share food resources with the five Corixidae species. Size-matched

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