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Repeated pulse exposures to lambda-cyhalothrin affect the behavior, physiology, and survival of the damselfly larvae *Ischnura graellsii* (Insecta; Odonata)

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ABSTRACT

Damselflies form an essential part of the aquatic and terrestrial food web. Pesticides may, however, negatively affect their behavior, physiology, and survival. To assess this, a 42-day-lasting bioassay was conducted, during which damselfly larvae (*Ischnura graellsii*; n = 20) were repeatedly exposed to lambda-cyhalothrin (3 days at; 0, 10, 50, 250, 1250, and 6250 ng LCH L^{-1}), followed by recovery phases (4 days) in pesticide-free medium for six weeks. This exposure design was used to simulate frequent runoff events in the field. Variables related to the behavior (strikes against prey and capture success), growth, physiology (lipid content and fatty acid composition), as well as mortality were assessed throughout the experiment. The two highest LCH concentrations induced 100% mortality within the first 48 h, whereas 85% of the test organisms survived 28 days under control conditions. The number of strikes against prey was not affected by LCH. In contrast, prey capture success decreased significantly (up to ~50% at 250 ng LCH L^{-1}, for instance, after the third pulse exposure) following LCH-exposures compared to the control. This difference was not observed after recovery phases, however, which did not counteract the enhanced energy demand for detoxification and defense mechanisms indicated by a lower growth rate (up to ~20%) and lipid content (up to ~30%) of damselflies at 50 and 250 ng LCH L^{-1}. In addition, two essential fatty acids (eicosapentaenoic acid and arachidonic acid) and two precursors (linolenic acid and α-linolenic acid) decreased in their concentrations upon exposure towards 250 ng LCH L^{-1}. Thus the results of this study indicate that long-term exposure towards LCH pulses can affect damselfly behavior, physiology and survival. Given the essential role of damselflies in food web dynamics, these effects may potentially translate into local population impairments with subsequent bottom-up directed effects within and across ecosystem boundaries.

1. Introduction

Pesticides that are applied for crop protection on agricultural fields can eventually enter surface waters via spray-drift or runoff during heavy rainfall events (Schulz, 2004). There, they are usually detected as pesticide mixtures (Bundschuh et al., 2014), within which the insecticide class of pyrethroids often dominates the mixtures' ecotoxicological potential. Ultimately, pyrethroids threaten the ecological integrity of exposed surface water bodies (Rasmussen et al., 2013; Weston et al., 2013; Wiberg-Larsen et al., 2016) inducing high risks for aquatic invertebrates (Halstead et al., 2015). Accordingly, multiple studies have documented adverse effects of pyrethroids on non-target organisms from the individual to the community level at environmentally relevant concentrations (e.g., Antwi and Reddy, 2015; Heckmann and Friberg, 2005; Schulz and Liess, 2000). However, the lipophilic properties of pyrethroids (Zhou et al., 1995) lead to short-term peak exposure (i.e., pulses) on organisms after the release of pesticides into surface waters. These peak exposures are characterized by concentrations of, for instance, lambda-cyhalothrin (LCH) of up to 500 ng L^{-1} (generally maximum concentrations are one or two orders of magnitude lower; Cooper et al., 2003). Peak exposure may only last for a few hours to days (Liess et al., 1999; Schulz et al., 1998; Leu et al., 2004). Subsequently, pyrethroid concentrations rapidly decrease due to adsorption to sediment particles and organic in-stream structures (Bennett et al., 2005; Hand et al., 2001), where they might still induce substantial negative effects in sediment dwelling organisms (Weston

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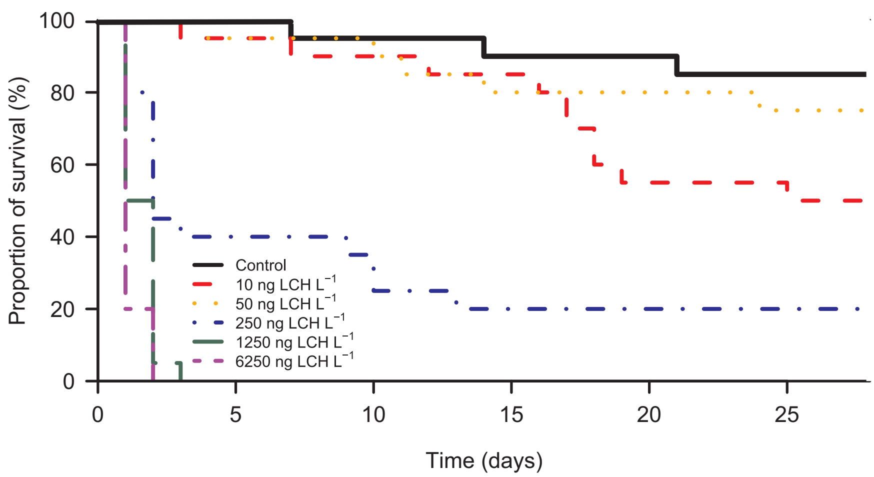

Fig. 1. Proportion of surviving (%) *I. graellsii* larvae during the first 28 days of the experiment, with lines representing the different LCH treatments (see in-figure legend).

et al., 2004). The rapid dissipation from the water phase in combination with frequent heavy precipitation – a pattern expected under projected climate change scenarios (Easterling et al., 2000) – and thus increasing surface runoff events, might cause repeated pulse exposures of aquatic organisms to pyrethroids. The ecotoxicological consequences of such repeated pulse exposures are, however, not well understood, though fundamental for a reliable estimation of pesticides' impact in aquatic life (Ashauer et al., 2007).

In surface waters, damselflies and dragonflies (Odonata) form an essential part of the aquatic food web before they emerge as adults to the adjacent terrestrial habitat (Stoks and Córdoba-Aguilar, 2012). Within the aquatic ecosystem, Odonata are predators of small invertebrates (e.g., mosquito larvae; Fincke et al., 1997) and prey for larger organisms (Stoks and Córdoba-Aguilar, 2012). Through their emergence, damselflies contribute to an energy flow from the aquatic to the terrestrial system that is available for terrestrial predators such as birds (Stoks and Córdoba-Aguilar, 2012). Hence, any alteration in their survival, growth, or physiology (e.g., essential fatty acid (FA) composition and consequently growth) may affect food web structure and the energy transfer within surface waters and across ecosystem boundaries (Schulz et al., 2015). At the same time, damselflies' aquatic habitats are under threat, with the majority of relevant factors being consequences of agricultural development (Clausnitzer, 2009). This in turn suggests that pesticides, in particular their most toxic representatives, are one potential factor for the threat.

Against this background, we conducted a 42-day bioassay during which damselfly larvae of the species *Ischnura graellsii* were repeatedly exposed to 3-day-lasting pulses of the model pyrethroid insecticide LCH (0, 10, 50, 250, 1250, and 6250 ng L^{-1}), followed by 4-day-long recovery phases in pesticide-free medium. This experimental design was thought to reflect an absolute worst-case exposure towards pyrethroids in terms of exposure frequency but also pulse exposure duration, with only limited possibilities to recover in-between pulse exposures while still simulating a field relevant scenario induced by frequent runoff events. The damselfly *I. graellsii* (Rambur, 1842) (Insecta, Odonata), which is endemic to the Iberian Peninsula and North Africa (Boudot and Kalkmann, 2015) as well as assessed as "least concern" in terms of its red list status (Kalkman et al., 2010), was used as model species. The habitat of the species is threatened by agricultural development and conservation action is needed (Clausnitzer, 2009), and larvae can be found clinging in the vegetation of standing and running waters (Aguesse, 1968). Both mortality and sublethal (i.e., lipid content, FA composition, growth rate, and prey capture behavior) effects on damselflies were assessed. We expected LCH to induce behavioral modifications as well as physiological effects at lower concentrations with increasing severity of effects with increasing LCH levels.

2. Materials and methods

2.1. Lambda-cyhalothrin concentrations and validation

LCH, purchased as analytical standard (PESTANAL®; purity ≥ 98%; Sigma-Aldrich, Seelze, Germany), was used as model pesticide. For the preparation of LCH stock solutions, the analytical standard was diluted in pure acetone given its low water solubility (5 µg L^{-1}, Lewis et al., 2016). Stock solutions were serially diluted in reconstituted soft water (i.e., test medium: for composition see Table S1; APHA, 1985), to achieve the final LCH concentrations of 0, 10, 50, 250, 1250, and 6250 ng L^{-1} shortly before the start of the experiment and the water exchanges (see section "Bioassay"), with a maximum share of 0.05% acetone in the test medium. Given the high 48 h LC_{50} of acetone reported for the standard test organism *Daphnia magna* (39 mL L^{-1}; LeBlanc and Surprenant, 1983) that corresponds to a share of 3.9% acetone in the test medium and lies approximately two orders of magnitude above the acetone share of the present study, no additional solvent control was conducted. The lower pesticide concentrations largely lay within the range of field measured LCH concentrations (up to 500 ng L^{-1}; Cooper et al., 2003). The higher concentrations were selected to ensure a full dose-response relationship and were based on empirical evidence obtained with the dragonfly *Erythromma viridulum* (Schroer et al., 2004).

The nominal test concentrations of LCH were validated for the control (0 ng L^{-1}) and the 250 ng L^{-1} treatment using a gas chromatography-mass spectrometry (GC-MS) system. The latter treatment represented the highest test concentration with surviving test organisms at the end of the bioassay (Fig. 1). For the chemical analysis, water samples were taken 10 min after pesticide application as well as just before the water exchange and stored at −20 °C. A volume of 250 mL from the control and 50 mL from the 250 ng LCH L^{-1} treatment was extracted, without prior filtration of the samples, using dichloromethane in a liquid-liquid extraction, while co-extracted water was eliminated using dry $NaSO_4$. Extracts were evaporated and reconstituted using cyclohexane:acetone in a 9:1 ratio (v:v) to 0.5 mL and 1.0 mL final volume for the control and the 250 ng LCH L^{-1} treatment, respectively. The GC-MS instrument consisted of a 7890 A gas chromatograph equipped with a temperature programmable injector (MMI) and connected to a 5975 C mass spectrometer with an ion source working in the negative chemical ionization (NCI) mode (Agilent technologies; Paola Alto, Ca, USA). The registered ion for LCH had a molecular mass/charge (m/z) of 240.9 and for the internal standard (Ethion) the m/z was 184.9. With a 3.0 µL injection volume the limit of detection for LCH was 0.1 ng L^{-1}. Since nominal and mean measured concentrations complied well with a maximum deviation of 6% (Table 1), an appropriate dosage of LCH is indicated that justifies the