



Strong differences between two congeneric species in sensitivity to pesticides in a warming world

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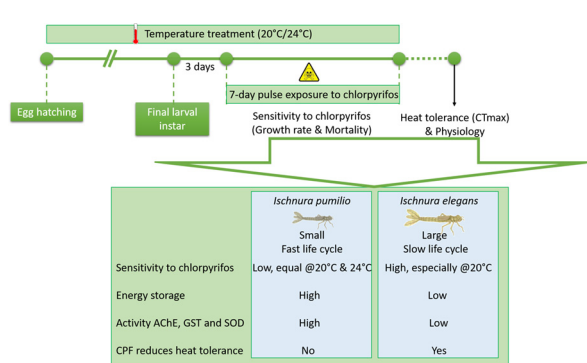
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HIGHLIGHTS

- Combined effects of warming and chlorpyrifos differed between two damselfly species.
- Chlorpyrifos reduced survival and growth more in the large, slow growing species.
- Chlorpyrifos reduced heat tolerance only in the more sensitive species.
- Differences in sensitivity were not driven by size and life history but physiology.
- Trait-based methods to predict sensitivity to pesticides need physiological traits

GRAPHICAL ABSTRACT



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ABSTRACT

To predict the impact of pesticides in a warming world we need to know how species differ in the interaction pathways between pesticides and warming. Trait-based approaches have been successful in identifying the 'pace of life' and body size as predictors of sensitivity to pesticides among distantly related species. However, it remains to be tested whether these traits allow predicting differences in sensitivity to pesticides between closely related species, and in the strength of the interaction pathways between pesticides and warming. We tested the effects of multiple pulses of chlorpyrifos (allowing accumulation) under warming on key life history traits, heat tolerance (CT_{max}) and physiology of two congeneric damselfly species: the fast-paced (fast growth and development, high metabolic rate), small *Ischnura pumilio* and the slow-paced, large *I. elegans*. Chlorpyrifos reduced survival and growth, but contrary to current trait-based predictions *I. pumilio* was 8× less sensitive than *I. elegans*. The lower sensitivity of *I. pumilio* could be explained by a higher fat content, and higher activities of acetylcholinesterase and of detoxifying and anti-oxidant enzymes. While for *I. pumilio* the effect of chlorpyrifos was small and did not depend on temperature, for *I. elegans* the impact was higher at 20 °C compared to 24 °C. This matches the higher pesticide accumulation in the water after multiple pulses at 20 °C than at 24 °C. The expected reduction in heat tolerance after pesticide exposure was present in *I. elegans* but not in *I. pumilio*. Our results demonstrate that closely related species can have very different sensitivities to a pesticide resulting in species-specific support for the "toxicant-induced climate change sensitivity" and the "climate-induced toxicant sensitivity" interaction pathways. Our results highlight that trait-based approaches can be strengthened by integrating physiological traits.

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1. Introduction

Pesticides and warming have the potential to interact, thereby challenging ecological risk assessment (ERA) of pesticides in a warming world (Noyes and Lema, 2015). On the one hand, the toxicity of many pesticides increases under warming (“climate-induced toxicant sensitivity”, Noyes and Lema, 2015). On the other hand, the ability to cope with high temperatures decreases under pesticide exposure (“toxicant-induced climate change sensitivity”, Hooper et al., 2013, Moe et al., 2013, Noyes and Lema, 2015). While both interaction pathways have large support, studies focused almost exclusively on the sensitivities of single species (but see Boeckman and Bidwell, 2006, Patra et al., 2007). To advance ERA in a warming world we need to know whether species predictably differ in these interaction pathways.

In general, ERA uses two different approaches to extrapolate sensitivity estimates from tested species to species with unknown sensitivity. In phylogeny-based approaches, the phylogenetic relatedness is used to infer similarity in sensitivity. The underlying assumption is that more related species have a more similar sensitivity (Hammond et al., 2012). In trait-based approaches, selected species traits are used as predictors to infer species sensitivity (Rubach et al., 2011; Van den Brink et al., 2011; Wiberg-Larsen et al., 2016). Life history traits, particularly the ‘pace of life’ and body size, have been put forward as important predictor traits. Species with a faster ‘pace of life’ have a faster growth and development, and a higher metabolic rate and are therefore expected to be more sensitive to stressors (Réale et al., 2010). This is expected for several reasons. Species that invest more energy in growth and development are expected to invest less in processes such as detoxification and damage repair because of energy-mediated trade-offs (Congdon et al., 2001). Moreover, species with a higher resting metabolic rate have been shown to be more sensitive to pesticides because of the associated higher turnover rate of pesticides, by which the toxic compounds can more easily interfere and cause damage (Baas and Kooijman, 2015). Further, smaller animals with a larger surface area to volume ratio have a higher uptake of pesticides leading to an increased sensitivity (Buchwalter et al., 2002; Rubach et al., 2012). Trait-based approaches have been successful in identifying the ‘pace of life’ and body size as predictors of sensitivity to pesticides among distantly related species (Rubach et al., 2012; Baas and Kooijman, 2015). However, it remains to be tested whether this trait-based extrapolation is also valid to predict differences in sensitivity between closely related species.

While at higher temperatures many pesticides (like organophosphates) become more toxic (Noyes et al., 2009), the extent to which the total impact of these pesticides will change under warming will depend on the relative changes in toxicity and exposure. The most commonly used method in ecotoxicology testing is static renewal, where the pesticide medium is daily refreshed. In these static renewal studies the sensitivity to pesticides typically increases with higher temperatures (e.g. Sokolova and Lannig, 2008; Dinh Van et al., 2014; Noyes and Lema, 2015). But under warming higher pesticide degradation rates may reduce exposure (Hooper et al., 2013), assuming that pesticide input remains constant (but see Kattwinkel et al., 2011). The higher degradation rates under warming can counter the increased toxicity of organophosphate pesticides under warming, potentially leading to a lower impact of pesticides at higher temperatures (Op de Beeck et al., 2017a, 2017b). Under realistic multiple pulse scenarios where pollutants can accumulate (Reinert et al., 2002) and that are predicted to become more frequent under warming (Kattwinkel et al., 2011), degradation rates will become especially important. Multiple pulse scenarios are therefore important to consider to arrive at a more realistic ERA of pesticides in a warming world. Yet, studies testing both interaction pathways between pesticides and warming using multiple pulse scenarios are rare (but see Op de Beeck et al., 2017b).

The main objectives of the current study were (i) to test how warming influenced the impact of multiple pulses of a pesticide, (ii) to investigate how these pesticide effects influenced the heat tolerance,

and (iii) to test whether sensitivity and the strength of both interaction pathways between warming and pesticides differed between closely related species with a different ‘pace of life’. To address these objectives, we focused on effects of warming and pesticide exposure on the damselfly *Ischnura pumilio* which has a fast ‘pace of life’ (rapid growth and development, high metabolic rate), and compared these effects with those on the closely related *I. elegans* which has a slow ‘pace of life’ (Debecker and Stoks, unpublished data). Based on the phylogeny-based approach we predict both species to show a similar sensitivity to the pesticide, yet based on the trait-based approach we expect the faster-living and smaller *I. pumilio* to be more sensitive to the pesticide than the slower-living and larger *I. elegans* (based on Réale et al., 2010, Rubach et al., 2012, Baas and Kooijman, 2015). To allow direct comparison, populations of both species were collected at the same latitude in Europe and tested simultaneously in the laboratory using a common garden experiment from the egg stage. The results for the *I. elegans* populations have been included in two previous papers where we focused on the comparison of pesticide effects under warming between *I. elegans* populations from different latitudes in Europe (Op de Beeck et al., 2017a, 2017b); these data here serve as direct comparison for the populations of *I. pumilio*. As pesticide we chose the organophosphate chlorpyrifos (CPF). CPF is one of the most frequently used pesticides worldwide (Eaton et al., 2008), situated within the top ten chemicals having the highest risk to organisms in surface waters in the UK (Johnson et al., 2017) and considered a priority pollutant in the European Water Framework Directive (2000/60/EC, Ojciec, 2000). Moreover, the trait-based studies that identified the ‘pace-of-life’ (Baas and Kooijman, 2015) and body size (Rubach et al., 2012) as predictors of sensitivity to pesticides explicitly studied exposure to CPF.

To study the impact of a multiple pulse application scenario of CPF under warming we focused on two key life history traits for damselflies (Stoks and Córdoba-Aguilar, 2012), mortality and growth rate, and on heat tolerance. As a measure of heat tolerance we determined the critical thermal maximum (CT_{max}), the upper temperature where animals no longer show body movements or muscular spasms (Verberk and Bilton, 2013). We looked at physiological mechanisms associated with effects on CT_{max}, by quantifying the activity of pyruvate kinase (PK) and lactate dehydrogenase (LDH), two enzymes related to aerobic/anaerobic energy production, and the amount of lactate as a metabolite of anaerobic respiration (Verberk et al., 2016). To further identify physiological mechanisms linked to the toxicity of CPF we also scored the activity of acetylcholine esterase (AChE), condition measures, and parameters related to oxidative stress and damage.

2. Methods

2.1. Collecting and housing

For each species, three randomly chosen populations in the northern region of the species’ areas were sampled. For *I. elegans* this were the Danish population Ahl Hage (56°10′59.66″N, 10°39′1.69″E) and the southern Swedish populations Nöbelövs mosse (55°44′6.00″N, 13°9′10.00″E) and Eriksö (58°56′4.90″N, 17°39′21.50″E). For *I. pumilio* this were the Danish populations Aarhus (56°6′30.20″N, 10°12′12.13″E), Grindsted (55°45′54.00″N, 8°58′16.00″E) and Tversted (57°36′27.86″N, 10°14′3.66″E). All populations occurred at shallow lakes with a rich aquatic vegetation. To equalize and minimize exposure to pesticides, all populations were chosen in natural areas with no surrounding agriculture (Coors et al., 2009; Cothran et al., 2013).

End June - early July 2014 twelve to eighteen mated females per population were collected and placed individually in small plastic vials with wet filter paper for oviposition. Eggs were transferred to the laboratory in Belgium. Throughout the experiment, eggs were incubated and larvae were reared at one of two water temperatures (20 °C or 24 °C) and a photoperiod of L:D 14:10 h. The lower temperature (20 °C) reflects the mean summer water temperature of ponds occupied by *I. elegans* at

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