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Extreme temperatures in the adult stage shape delayed effects of larval pesticide stress: A comparison between latitudes

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ABSTRACT

Global warming and pesticide pollution are major threats for aquatic biodiversity. Yet, how pesticide effects are influenced by the increased frequency of extreme temperatures under global warming and how local thermal adaptation may mitigate these effects is unknown. We therefore investigated the combined impact of larval chlorpyrifos exposure, larval food stress and adult heat exposure on a set of fitness-related traits in replicated low- and high-latitude populations of the damselfly *Ischnura elegans*. Larval pesticide exposure resulted in lighter adults with a higher water content, lower fat content, higher Hsp70 levels and a lower immune function (PO activity). Heat exposure reduced water content, mass, fat content and flying ability. Importantly, both stressors interacted across metamorphosis: adult heat exposure lowered the reduction of fat content, and generated a stronger decrease in PO activity in pesticide-exposed animals. Larval pesticide exposure and larval food stress also reduced the defense response to the adult heat stress in terms of increased Hsp70 levels. In line with strong life history differences in the unstressed control situation, high-latitude animals were less sensitive to food stress (body mass and water content), but more sensitive to pesticide stress (development time and PO activity) and heat exposure (PO activity and Hsp70 levels). While low-latitude adults could better withstand the extreme temperature as suggested by the weaker increase in Hsp70, heat exposure similarly affected the delayed effects of larval pesticide exposure at both latitudes. Our study highlighted two key findings relevant for ecological risk assessment under global warming. Firstly, the delayed effects of larval pesticide exposure on adult damselflies depended upon subsequent adult heat exposure, indicating that larval pesticide stress and adult heat stress interacted across metamorphosis. Secondly, low- and high-latitude animals responded differently to the imposed stressors, highlighting that intraspecific evolution along natural thermal gradients may shape sensitivity to pesticides.

1. Introduction

High temperatures are receiving increased attention in ecotoxicology as these typically increase the toxicity of contaminants such as pesticides (Noyes et al., 2009) and therefore are critical when extrapolating toxicity tests across seasons and along thermal gradients (Clements et al., 2012). Especially the need to understand the impact of pesticides under global warming generated a surge of studies (Landis et al., 2013; Moe et al., 2013). These studies generated very valuable information for the development of a predictive framework for ecological risk assessment along natural thermal gradients and under global warming.

Studies on global warming typically exposed organisms to a moderate temperature increase, as predicted by relevant warming scenarios (IPCC, 2007). Such moderate temperature increases are often beneficial for temperate organisms in the absence of contaminants (Deutsch et al., 2008; Stoks et al., 2014). Global warming will, however, also be characterized by more frequent temperature extremes (IPCC, 2012) and these are more likely to negatively impact organisms (Jentsch et al., 2007). Yet, temperature extremes have received much less attention in ecotoxicology (but see Kaur et al., 2011).

While most studies simultaneously exposed organisms to contaminants and warming, there is increasing concern that stressors may interact in a delayed way (Segner, 2011). Such delayed effects are easily overlooked in the many animals that have a complex life cycle with a larval aquatic stage and an adult, terrestrial stage, separated by metamorphosis (Moran, 1994). Several studies have documented carry-over effects of larval pesticide exposure on adult traits (e.g. Campero et al., 2008; Distel and Boone, 2010; Boone et al., 2013), but few have explored how exposure to contaminants

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in the larval stage affects the sensitivity of the animals to a different stressor in the adult stage (e.g. Rohr and Palmer, 2005) and no studies so far did this in a global warming framework and tested how delayed effects of larval pesticide exposure are shaped by adult exposure to extreme temperatures.

Another aspect that may further increase realism to assess the impact of contaminants under global warming is the consideration of thermal adaptation shaping the sensitivity to contaminants at higher temperatures (Moe et al., 2013). Given that temperature extremes are more frequent at lower latitudes (Orlowsky and Seneviratne, 2012), it is to be expected that low-latitude populations may suffer less from extreme temperatures (Hoffmann et al., 2002). This generates the hypothesis that temperature extremes will differentially affect the carry-over effects of larval pesticide stress in low- and high-latitude populations.

We addressed all three above-mentioned issues by investigating the combined impact of larval sublethal pesticide exposure and subsequent adult exposure to extreme temperatures on a set of fitness-related traits in a common garden experiment with replicated populations at the lower and higher part of the latitudinal range of an aquatic insect. Given their limited ability to escape exposure, many ectothermic aquatic organisms are particularly vulnerable to combinations of contaminants and high temperatures (Bronmark and Hansson, 2002; Woodward et al., 2010). We additionally manipulated food stress in the larval stage, because this may make energy-mediated carry-over effects more apparent (Karl et al., 2011). Moreover, suboptimal food conditions may increase the impact of pesticide stress (Janssens and Stoks, 2013a) and of exposure to extreme temperatures (Adamo et al., 2012). As study species we chose the damselfly *Ischnura elegans* and tested animals originating from different latitudes, hence with a different evolutionary history of heat extremes. Damselflies are especially sensitive to global warming (Hassal and Thompson, 2008) and given their aquatic larval stage and terrestrial adult stage, stressor effects after metamorphosis may couple aquatic and terrestrial ecosystems (Stoks and Cordoba-Aguilar, 2012). We quantified the following fitness-related traits in damselflies (Stoks and Cordoba-Aguilar, 2012): survival, emergence success and development time in the larval stage and flying ability, body mass, water content, fat content, levels of the stress protein Hsp70 and phenoloxidase activity in the adult stage. As model pesticide we chose chlorpyrifos, an organophosphate insecticide that functions as an inhibitor of acetylcholinesterase (Stenersen, 2004). Chlorpyrifos is one of the most used pesticides worldwide (Eaton et al., 2008) and is often found in ponds where it negatively affects non-target organisms, including aquatic insects (Rubach et al., 2012).

2. Methods

2.1. Collecting and housing

We studied populations of the damselfly *I. elegans* from two latitudes representing the low-latitude (southern France) and high-latitude (southern Sweden) parts of the range in Europe (Gosden et al., 2011). At each latitude three randomly chosen populations were sampled, namely Saint-Martin de Crau (+43°37′57.88″N, +4°46′55.18″E), Laune des Irudes (+43°30′34.80″N, +4°48′03.83″E) and Bassin de Réaltor (+43°28N11.16, +5°19′44.16″E) for France and Kalmar Dämme (+56°40′9.84″N, 16°17′48.48″E), Svino (+56°41′4.32″N, +16°22′23.79″E) and Hougårdsdammarna (+57°15′1.78″N, +12°8′1.983″E) for Sweden. These populations were chosen in natural areas without agriculture and it is therefore unlikely that they were exposed to pesticides (Coors et al., 2009). Furthermore, any local adaptation to pesticides would be unlikely in coenagrionid damselflies given their high levels of gene flow (Johansson et al., 2013). At both latitudes temperatures above 30 °C have been documented, yet such hot days are much more frequent in southern France compared to southern Sweden. While in southern France (weather station Tour-du-Valat) the average number of days with air temperatures equal to or above 30 °C equaled 34 days per year during the period 1992–2008, this average was only one day per year in southern Sweden (weather station Malmö).

Eggs of fifteen mated females per population were collected and transferred to the laboratory in Belgium. One week after hatching, larvae were placed individually in 200 ml cups. Throughout the experiment, larvae were reared in incubators at 20 °C and L:D 14:10 h. Larvae were daily fed *ad libitum* with *Artemia* nauplii five days a week (average daily dose = 1347 nauplii, SE = 102, n = 15 daily doses, each dose collected on a different day).

2.2. Pesticide concentration

Based on a previous experiment, we used pulses of 2.0 μg/l chlorpyrifos, since this causes a growth reduction and only limited mortality in *I. elegans* damselfly larvae (Dinh Van et al., in prep.). This concentration may be very high in general, but not in agricultural water bodies preferred by the study species (Dijkstra, 2006). Here peak concentrations often exceed 100 μg/l due to runoff (e.g. Moore et al., 2002; Mazanti et al., 2003; Bernabo et al., 2011).We prepared the chlorpyrifos solution starting from a stock solution with a concentration of 20 μg/ml chlorpyrifos (kept in the dark at 4 °C). This stock solution was a 50 times dilution of another stock solution containing 1 mg/ml chlorpyrifos dissolved in ethanol. The chlorpyrifos concentration of the stock solution at the start and at the end (3 months later) of the experiment was 1.000 mg/ml and 0.975 mg/ml, respectively. Samples were analyzed by the independent research laboratory Lovap NV (Geel, Belgium) using gas chromatography in combination with mass spectrometry. The initial chlorpyrifos concentration in the experimental vials was 2.020 μg/l and after three days (just before renewal of the medium) the concentration was lowered to 0.884 μg/l, indicating that although the chlorpyrifos concentration fluctuated in time, the larvae were continuously exposed to the pesticide.

We used aerated dechlorinated tap water in the control treatment instead of a solvent control since the amount of ethanol was only 2 μl/l exposure medium. A pilot experiment showed that there was no difference in survival, growth and development time of the study species at this ethanol concentration (Janssens, unpublished results). Moreover, the lowest NOEC reported for aquatic invertebrates is >10,000 times higher than the ethanol concentrations used in the pesticide treatment (UNEP, 2004).

2.3. Experimental setup

To test for effects of larval pesticide exposure, larval food stress and adult heat exposure on survival, life history and physiological variables in *I. elegans* damselflies and compare those between two different latitudes, we set up a full factorial experiment with two latitudes (France versus Sweden, with three replicated populations per latitude) × two food regimes (high food level versus low food level) × three pesticide conditions (control, 16 days exposure, complete final instar exposure) × two adult heat conditions (20 °C versus 30 °C) giving a total of 24 treatment combinations. We set up 35 larvae per treatment combination (total of 840 larvae). Due to differential mortality, sample sizes per response variable differ among treatment combinations and are shown in the figures.

The day after the larvae molted into the final instar (=F_0 stage), they entered the experiment. We used two different food levels: animals were fed *Artemia* nauplii twice a day six days a week (high