Aquatic Toxicology 167 (2015) 38–45

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Warmer winters modulate life history and energy storage but do not affect sensitivity to a widespread pesticide in an aquatic insect

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ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form 9 July 2015
Accepted 28 July 2015
Available online 31 July 2015

Keywords:
Damselfly larvae
Chlorpyrifos exposure
Temperature increase
Thermal adaptation

ABSTRACT

Despite the increased attention for the effects of pesticides under global warming no studies tested how winter warming affects subsequent sensitivity to pesticides. Winter warming is expected to cause delayed negative effects when it increases metabolic rates and thereby depletes energy reserves. Using a common-garden experiment, we investigated the combined effect of a 4 °C increase in winter temperature and subsequent exposure to chlorpyrifos in the aquatic larvae of replicated low- and high-latitude European populations of the damselfly *Ischnura elegans*. The warmer winter (8 °C) resulted in a higher winter survival and higher growth rates compared to the cold winter (4 °C) commonly experienced by European high-latitude populations. Low-latitude populations were better at coping with the warmer winter, indicating thermal adaptation to the local winter temperatures. Subsequent chlorpyrifos exposure at 20 °C induced strong negative effects on survival, growth rate, lipid content and acetylcholinesterase activity while phenoloxidase activity increased. These pesticide effects were not affected by winter warming. Our results suggest that for species where winter warming has positive effects on life history, no delayed effects on the sensitivity to subsequent pesticide exposure should be expected.

1. Introduction

Pollution and global warming are two key threats to biodiversity (Millenium Ecosystem Assessment, 2005). Moreover, both threats are expected to interact with each other. One emerging insight is that many contaminants are more toxic at higher temperatures (Noyes et al., 2009; Holmstrup et al., 2010). This insight comes from studies that directly exposed organisms to contaminants at higher temperatures. There is, however, increasing concern that contaminants may show delayed effects and that these delayed effects interact with stressors that are subsequently imposed (Segner, 2011). Such delayed effects have been less studied in ecotoxicology, particularly in the context of global warming (but see Janssens et al., 2014; Arambourou and Stoks, 2015).

Another largely ignored aspect at the interplay of ecotoxicology and global change biology is the effect of warmer winters on the subsequent sensitivity to contaminants. While most attention in global change biology has gone to the effects of increasing summer temperatures, fewer studies have focused on the effects of increasing winter temperatures. This is relevant as the strongest effect of global warming is not on increasing summer heat but on reducing winter cold (Bradshaw and Holzapfel, 2008). The limited understanding of organismal responses to winter therefore impedes prediction of the biological impacts of global warming (Williams et al., 2015). While relatively much is known about the effects of winter warming on terrestrial organisms (reviewed by Williams et al., 2015) this is less so for aquatic ectotherms (but see Hogg et al., 1995). For aquatic ectotherms at northern latitudes an important factor to consider is the shortened duration of ice cover under global warming (Manugson et al., 2000).

Based on research in terrestrial ectotherms, winter warming may have two opposing effects. On the one hand, winter warming may be beneficial as during cold winters energetic deficits may accrue and lead to mortality (Hahn and Denlinger, 2011). On the other hand, winter warming may be detrimental as many ectotherms rely on low winter temperatures to reduce consumption of energy stores (e.g. Irwin and Lee, 2003; Williams et al., 2012b). The reason why some species benefit and other species suffer from winter warming is poorly understood and even congeneric species may differ in their response (Williams et al., 2012a). Given the potential opposing effects of winter warming on energy deficits (Williams et al., 2012a), the delayed effects of winter warm-

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http://dx.doi.org/10.1016/j.aquatox.2015.07.018
0166-445X/

ing on subsequent spring/summer applications of pesticides needs empirical testing. Given that detoxification is energetically costly (Sibly and Calow 1989; Congdon et al., 2001), any energy deficits accumulated during winter that are not remediated before the spring/summer pesticide applications will likely increase the sensitivity to pesticides.

An added complexity in the study of contaminants under global warming is that local thermal adaptation may mediate the effects of pesticides at higher temperatures (Dinh Van et al., 2013; Moe et al., 2013). Given that populations at lower latitudes experience higher winter temperatures they are likely better adapted to higher winter temperatures than populations at higher latitudes and vice versa. Comparing the effects of contaminants between low- and high-latitude populations that have undergone simulated warm and cold winters allows us to assess the role of thermal adaptation in shaping the sensitivity to contaminants. Furthermore, the sensitivity to contaminants of low-latitude populations reared at their current warm winter temperatures may be used as a proxy to estimate the predicted sensitivity to contaminants of high-latitude populations under the predicted winter warming by 2100 (assuming gradual thermal evolution to the warmer winter temperatures). Few studies (but see Dinh Van et al., 2013; Janssens et al., 2014) have applied this so-called space-for-time substitution approach (Fukami and Wardle, 2005) in ecotoxicology, and none of these studies have considered the effects of winter warming.

In the present study, we investigated how winter warming affects life history and the sensitivity to a subsequent exposure to the widespread pesticide chlorpyrifos (CPF) at the spring/summer temperature in larvae of an aquatic insect. Moreover, to evaluate the potential of local thermal adaptation mediating the effects of warmer winters, we compared these effects between replicated high- and low-latitude populations. CPF, an organophosphate insecticide, is a priority pollutant in the European Water Framework Directive (2000/60/EC). As study species we chose *Ischnura elegans* (Odonata, Coenagrionidae) a very common damselfly species in Europe with a broad latitudinal range (Gosden et al., 2011) whose response to CPF has been well studied (e.g., Dinh Van et al., 2014; Janssens et al., 2014; Arambourou and Stoks, 2015). Damselfly larvae are particularly sensitive to global warming (Hassall and Thompson, 2008) and to organic toxicants (Liess and von der Ohe, 2005) and proven to be elegant study organisms to address ecological and evolutionary questions in ecotoxicology (Stoks et al., 2015). We measured effects on two life-history traits (mortality and growth rate) and three fitness-related biochemical markers: lipid content, the activity of the enzyme acetylcholinesterase (AChE) and the activity of the enzyme phenoloxidase (PO). Because organophosphate insecticides act as AChE inhibitors, AChE activity has been widely used as a biomarker of CPF exposure (Fulton and Key, 2001). As detoxification is energetically costly, the lipid content can be reduced by pesticide exposure (Janssens and Stoks, 2013). Because a previous study has shown an activation of PO activity in response to CPF exposure (Arambourou and Stoks, 2015), we also measured PO activity, a key enzyme of the insect immune system (González-Santoyo and Córdoba-Aguilar, 2012).

2. Material and methods

2.1. Test animals and culture conditions

During the summer of 2013, *I. elegans* females were collected in the field at the high- and the low-latitude parts of the species' range in Europe (Gosden et al., 2011). The high-latitude populations were sampled at Eriksö (59°39′N, 17°34′E) and Lund (55°70′N, 13°03′E), both situated in southern Sweden and separated by 490 km. We will refer to them as SWER and SWLU, respectively. The low-latitude populations were sampled in Spain (43°29′N, 8°18′W) and in France (43°38′N, 4°49′E). These populations are separated by 1070 km. We will refer to them as SP and FR, respectively. The north–south distance between the two latitudes is approximately 1500 km. Sampled populations come from shallow water bodies located in low urbanized areas without cropland. Therefore, it is unlikely that these populations are exposed to pesticides (Coors et al., 2009) and have gained a resistance against pesticides (Hua et al., 2015).

Field-collected females were placed individually in small plastic containers and given wet filter paper as oviposition substrate in the laboratory. Between 7 and 10 egg clutches from different females per population were transported to the laboratory in Belgium. They were then placed in plastic tanks in an incubator at 20 ± 1 °C on a 14:10 h light:dark photoperiod with a light intensity of 6000 ± 1000 lux. Aged dechlorinated tap water was used as culture water. We monitored egg clutches daily for hatching. Between 10 and 15 days after hatching, each larva was placed individually in a circular plastic 180 ml cup filled to a height of 5 cm. Larvae were fed five days a week ad libitum with nauplii of *Artemia salina.*

2.2. CPF concentration

The CPF stock solution was prepared by adding 2 mg of CPF (Pestanal®, analytical standard) in 2 ml of absolute ethanol (Normapur®, VWR). This solution was then diluted in aged dechlorinated tap water to obtain a solution at a nominal concentration of 2 μg/l. The studied concentration is higher than the concentrations commonly measured in surface waters (Murray et al., 2010; Navarro et al., 2010; Reilly et al., 2012) but can be detected in water bodies in agricultural areas (Zhang et al., 2012). To prepare the control solution, the same procedure was used with corresponding concentrations of ethanol diluted in aged dechlorinated tap water.

2.3. Experimental design

We exposed damselfly larvae from the two high- and the two low-latitude populations of *I. elegans* either to a normal cold high-latitude winter or to a simulated warm high-latitude winter followed by an exposure to CPF under simulated common spring/summer conditions. This setup simulates the effect of winter warming at the high latitude and tests how this affects the sensitivity to CPF during the next spring/summer. Considering that in southern Sweden ponds are covered by ice during winter for approximately 54–119 days (Blenckner et al., 2002), and that the water temperature under the ice is approximately 4 °C (De Block et al., 2007), we kept larvae during the normal cold winter for 90 days at 4 °C. For the simulated warm winter, an increase of 4 °C was considered – based on the IPCC scenario RCP 8.5 (IPCC, 2013) – hence these larvae were kept for 90 days at 8 °C. During winter the water temperature is on average 6 °C in the French area and 10 °C in the Spanish area (based on Flake-model simulations performed on the 1998–2012 period, see Dinh Van et al., 2014 for the detailed methodology).

The experiment was fully factorial with 16 treatment groups: 2 replicated populations per latitude × 2 latitudes × 2 winter temperature treatments (4 °C or 8 °C) × 2 pesticide treatments (CPF absent or present). Given that larvae are not exposed to pesticide pulses during winter, we only exposed larvae to the pesticide after the simulated winter. Larvae were exposed to the pesticide at 20 °C, which is an often encountered spring temperature and the mean summer water temperature in the shallow ponds occupied by the study species at the high latitude (see De Block et al., 2013). At the