Environmental Pollution 230 (2017) 351–359

Contents lists available at ScienceDirect

Environmental Pollution

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

Testing the time-scale dependence of delayed interactions: A heat wave during the egg stage shapes how a pesticide interacts with a successive heat wave in the larval stage☆

Lizanne Janssens*, Nedim Tüzün, Robby Stoks

Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Charles Deberiotstraat 32, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 15 May 2017
Received in revised form
20 June 2017
Accepted 24 June 2017
Available online 29 June 2017

Keywords:
Delayed pesticide effects
Global warming
Multistressor studies
Pyrethroid
Stressor interactions
Survival selection

ABSTRACT

Under global change organisms are exposed to multiple, potentially interacting stressors. Especially interactions between successive stressors are poorly understood and recently suggested to depend on their timing of exposure. We particularly need studies assessing the impact of exposure to relevant stressors at various life stages and how these interact. We investigated the single and combined impacts of a heat wave (mild [25 °C] and extreme [30 °C]) during the egg stage, followed by successive exposure to esfenvalerate (ESF) and a heat wave during the larval stage in damselflies. Each stressor caused mortality. The egg heat wave and larval ESF exposure had delayed effects on survival, growth and lipid peroxidation (MDA). This resulted in deviations from the prediction that stressors separated by a long time interval would not interact: the egg heat wave modulated the interaction between the stressors in the larval stage. Firstly, ESF caused delayed mortality only in larvae that had been exposed to the extreme egg heat wave and this strongly depended upon the larval heat wave treatment. Secondly, ESF only increased MDA in larvae not exposed to the egg heat wave. We found little support for the prediction that when there is limited time between stressors, synergistic interactions should occur. The intermediate ESF concentration only caused delayed mortality when combined with the larval heat wave, and the lowest ESF concentrations only increased oxidative damage when followed by the mild larval heat wave. Survival selection mitigated the interaction patterns between successive stressors that are individually lethal, and therefore should be included in a predictive framework for the time-scale dependence of the outcome of multistressor studies with pollutants. The egg heat wave shaping the interaction pattern between successive pesticide exposure and a larval heat wave highlights the connectivity between the concepts of 'heat-induced pesticide sensitivity' and 'pesticide-induced heat sensitivity'.

1. Introduction

The growing concern that animals will not only have to cope with warming, but also with the changed impact of pollutants in a warming world boosted research at the interface of global change biology and ecotoxicology (Moe et al., 2013; Noyes and Lema, 2015). In the field of global change biology there is a strong bias toward research on mild warming, while effects of heat waves have been understudied (Thompson et al., 2013). Recent insights indicate, however, that the effects of heat waves may be stronger and may overrule the effects of mild warming (Jentsch et al., 2007; Williams et al., 2016). This bias towards mild warming studies is also present in ecotoxicology. Nevertheless, the number of combined studies on pollutants and extreme temperatures as experienced during heat waves is increasing (e.g. Chang et al., 2007; Holmstrup et al., 2010; Arambourou and Stoks, 2015). This follows the general insight that we need more studies that consider interactions between stressors to improve the risk assessment of pollutants and conservation management in general (Côté et al., 2016; Liess et al., 2016).

Especially interaction patterns between successive stressors are poorly understood and suggested to depend on their timing of exposure (Ashauer et al., 2010; Gunderson et al., 2016). The majority of studies exposing animals to heat waves and pollutants did so simultaneously with the general outcome of a stronger, synergistic impact of pollutants under extreme temperatures (Holmstrup

☆ This paper has been recommended for acceptance by Dr. Chen Da.

* Corresponding author.
E-mail address: Lizanne.janssens@kuleuven.be (L. Janssens).

http://dx.doi.org/10.1016/j.envpol.2017.06.082
0269-7491/

et al., 2010). Simultaneous exposure to two stressors is indeed more likely to generate synergistic interactions since the physiological response to the first stressor is still being mounted when the second stressor occurs, resulting in no toxicodynamic recovery (Ashauer et al., 2010). Yet, as animals typically encounter heat waves (Meehl and Tebaldi, 2004) and pesticides (Van Drooge et al., 2001) only for limited periods of time, animals will be more likely exposed successively to both stressors. When two stressors are in close succession, the physiological response to the first stressor may still be ongoing, and if most of the damage is repaired or removed before the second stressor occurs, this may generate cross-resistance and antagonistic interactions (Gunderson et al., 2016). When stressors are instead spread over a long timescale, no interactive effects are expected since the organisms are thought to have returned to their homeostatic phase before the second stressor occurs (Gunderson et al., 2016). Alternatively, exposure to one stressor can lead to a permanent increase of an organism's baseline defence levels, making it easier for it to cope with future stressors (Monaghan et al., 2009).

Crucial to advance our insights into how heat waves will interact with pollutants, are studies that expose animals successively to these stressors. We particularly need studies assessing the impact of exposure to relevant stressors at various life stages and how these interact. These will be essential to test the conceptual framework of Gunderson et al. (2016) and to address other questions related to the timing of exposure to stressors. Many organisms will be exposed to more complex stressor combinations and one outstanding question is whether exposure to one stressor early in life may change the way two other stressors later in life interact with each other. Given the rarity of three-stressor studies in ecotoxicology in general (but see e.g. Przeslawski et al., 2005; Coors and De Meester, 2008; Matthaei et al., 2010; Dinh Van et al., 2016a), this has been unexplored. Nevertheless, such studies will be crucial to advance our insights in the still poorly understood occurrence of interactions between pollutants and natural stressors (Crain et al., 2008; Liess et al., 2016).

While there is increasing appreciation that stressors may interact in a delayed way (Segner, 2011; Gunderson et al., 2016), the studies integrating stress exposure in two life stages typically consider the larval stage and the adult stage (e.g. Rohr and Palmer, 2005; Janssens et al., 2014). In contrast, delayed effects of stressors experienced in the egg stage on traits later in the development, and especially their interaction with stressors occurring later in life have been rarely studied (but see Broomhall, 2004; Zhang et al., 2014; Sniegula et al., 2017). Such studies are, however, highly relevant as in the egg stage organisms are less mobile and therefore do not have the possibility to avoid exposure to stressors (Potter et al., 2011).

In this study we investigated the single and combined effects of a heat wave during the egg stage, followed by successive exposure to a pyrethroid pesticide and a heat wave during the larval stage of a damselfly. The study species, *Lestes viridis*, deposits eggs in summer, which will hatch the following spring; making a heat treatment for the eggs and pesticide exposure and a heat treatment for the larvae, relevant stressors to study in these different life stages. Special focus was on the delayed effects of the stressors and how a stressor in the egg stage interacted with stressors encountered later in life. Based on Gunderson et al. (2016) we may expect few effects of the egg heat wave on the impact of the larval pesticide exposure and the larval heat wave, while the latter two are expected to interact in a synergistic way because they are in close succession. We studied effects on survival and growth, and an important physiological variable linked with life history and performance, oxidative damage to lipids (Monaghan et al., 2009). Oxidative stress is thought to be a key molecular mechanism involved in pyrethroid-induced toxicity (e.g. Rodrigues et al., 2015). As pyrethroid pesticide we chose the insecticide esfenvalerate (ESF), one of the most widely applied pyrethroids (Beketov and Liess, 2005; Spurlock and Lee, 2008). Pyrethroids function by damaging the nervous system through interference with ion channels in the nerve axons (Tomlin, 2001). We chose damselflies as study organisms since they are particular vulnerable in the aquatic larval stage to both global warming (Hassall and Thompson, 2008) and to ESF exposure (Beketov, 2004).

2. Methods

2.1. Collection and housing

Lestes viridis oviposits in tree twigs overhanging ponds. Eggs of this species are laid in summer, enter a winter diapause and hatch from April to half June in the study region (De Block et al., 2005), with a hatching peak at the end of April (De Block and Stoks, 2004). The duration of the larval period is ca. 65 days (De Block and Stoks, 2003). Twigs containing eggs of *L. viridis* were collected from a population in Edegem (51°09′19.1″N, 4°23′55.6″E), Belgium on 27 January 2015. Twiggs with eggs can be easily recognized by the gall-like structures where the eggs have been deposited, and the oviposition marks are easily visible (each mark typically is associated with 4 eggs). The population was chosen in an area without agriculture and therefore is unlikely to be exposed to pesticides (Coors et al., 2009). The twigs with eggs were placed in incubators in trays filled with dechlorinated tap water. The eggs were gradually warmed (10 °C on 28 January, 15 °C on 1 February, 20 °C on 5 February). After hatching, groups of 10 larvae were placed together in 200 ml plastic cups filled with dechlorinated tap water in water baths (20 °C, 14:10 L:D) to enhance survival (De Block and Stoks, 2003). Two weeks after hatching, larvae were placed individually in the same cups. During their life, the larvae were fed *ad libitum* with *Artemia* nauplii.

2.2. Experimental setup

To test for the single and combined effects of subsequent exposure to a heat wave in the egg stage ('egg heat wave'), pesticide exposure in the larval stage and a heat wave in the larval stage ('larval heat wave'), we set up a full factorial experiment (Fig. 1). To test how one stressor early in life may change the way two other stressors later in life interact with each other, the egg heat wave

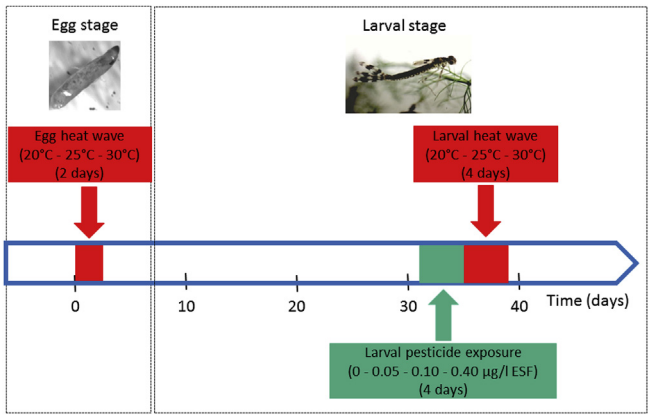

Fig. 1. Experimental design with indication of the timing of exposure to the three successive stressors: the egg heat wave, the larval pesticide exposure and the larval heat wave. Day zero was defined as the day when the first stressor treatment (the egg heat wave) started.