Aquatic Toxicology 167 (2015) 20–30

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Choice of experimental venue matters in ecotoxicology studies: Comparison of a laboratory-based and an outdoor mesocosm experiment

Zsanett Mikó[a,*], János Ujszegi[a], Zoltán Gál[a], Zoltán Imrei[b], Attila Hettyey[a]

[a] Lendület Evolutionary Ecology Research Group, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman Ottó út 15, Budapest 1022, Hungary
[b] Applied Chemical Ecology Department, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman Ottó út 15, Budapest 1022, Hungary

ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 16 June 2015
Accepted 24 July 2015
Available online 29 July 2015

Keywords:
Experimental venue
Herbicide
Life-history trait
Multiple stressors
Predator
Rana dalmatina tadpole

ABSTRACT

The heavy application of pesticides and its potential effects on natural communities has attracted increasing attention to inadvertent impacts of these chemicals. Toxicologists conventionally use laboratory-based tests to assess lethal concentrations of pesticides. However, these tests often do not take into account indirect, interactive and long-term effects, and tend to ignore different rates of disintegration in the laboratory and under natural conditions. Our aim was to investigate the importance of the experimental venue for ecotoxicology tests. We reared tadpoles of the agile frog (*Rana dalmatina*) in the laboratory and in outdoor mesocosms and exposed them to three initial concentrations of a glyphosate-based herbicide (0, 2 and 6.5 mg a.e./L glyphosate), and to the presence or absence of caged predators (dragonfly larvae). The type of experimental venue had a large effect on the outcome: The herbicide was less lethal to tadpoles reared in outdoor mesocosms than in the laboratory. Further, while the herbicide had a negative effect on development time and on body mass in the laboratory, tadpoles exposed to the herbicide in mesocosms were larger at metamorphosis and developed faster in comparison to those reared in the absence of the herbicide. The effect of the herbicide on morphological traits of tadpoles also differed between the two venues. Finally, in the presence of the herbicide, tadpoles tended to be more active and to stay closer to the bottom of laboratory containers, while tadpole behaviour shifted in the opposite direction in outdoor mesocosms. Our results demonstrate major discrepancies between results of a classic laboratory-based ecotoxicity test and outcomes of an experiment performed in outdoor mesocosms. Consequently, the use of standard laboratory tests may have to be reconsidered and their benefits carefully weighed against the difficulties of performing experiments under more natural conditions. Tests validating experimentally estimated impacts of herbicides under natural conditions and studies identifying key factors determining the applicability of experimental results are urgently needed.

1. Introduction

Biodiversity is declining worldwide at an accelerating rate, so that identifying the complex causes of species extinctions has become one of the greatest challenges in ecology (May, 2010; Pereira et al., 2010). The major causes of these declines are habitat loss and fragmentation, but further causes have been put forward recently, including climate change, increasing UV-B radiation, invasive or spreading predators, competitors and parasites, emerging diseases and the heavy use of pesticides (e.g., Blaustein and Kiesecker, 2002; Clausen and York, 2008; Clavero et al., 2009; Dirzo and Raven, 2003; Hayes et al., 2010; Hof et al., 2011).

The application of pesticides is an effective way of improving productivity in agriculture and the advantages of pesticide use are well documented (Jones et al., 2010). However, pesticides can affect physiology and decrease reproductive success, disrupt endocrine functions and have immunotoxic effects not only in pests, but also in non-target organisms as well (Albers, 2003; Colborn et al., 1996; O'shea and Tanabe, 2003; Ratcliffe, 1967). Hence, pesticides and their residues can negatively impact persistence of species and, ultimately, biodiversity (Sotherton and Holland, 2003). What adds to the problem is that many users do not have sufficient

* Corresponding author. Fax: +36 13918653.
E-mail address: miko.zsanett@agrar.mta.hu (Z. Mikó).

http://dx.doi.org/10.1016/j.aquatox.2015.07.014
0166-445X/

knowledge about the possible risks, the optimal application methods and the necessary precautions that need to be considered when applying pesticides (Damalas and Eleftherohorinos, 2011). These factors together not only result in serious health hazards for humans, but can also threaten non-target species and entire ecosystems (Berny, 2007).

Studies concerned with the effects of pesticides on non-target organisms can be divided into two groups; acute and chronic toxicity tests. Acute tests are traditionally designed for estimating LC50 or LD50, the medial lethal concentration or dose that kills half of the members of a population of a model species. Chronic tests, on the other hand, not only evaluate survival, but also sublethal effects on behaviour, growth and reproduction, and cover more than ten percent of the lifetime of the studied organisms (Adams and Rowland, 2003; Suter, 2007). The estimates on the potential impacts of pesticides determined under standard laboratory conditions are then used to determine environmentally safe concentrations of pollutants (Sih et al., 2004). Such standardized laboratory tests can have high throughput, their results can easily be interpreted and compared among laboratories, and they often correctly predict lethal or sublethal toxic effects on natural communities (Chalcraft et al., 2005; Versteeg et al., 1999). However, this approach usually does not take into account additive or synergistic effects of multiple biotic and abiotic stress factors (Sih et al., 2004). Also, standard laboratory tests typically neglect indirect effects, although top–down or bottom–up trophic cascades, keystone predation, competition or indirect facilitation may all modulate realized effects of a contaminant (Relyea and Hoverman, 2006). Finally, it has been shown that ecological experiments can yield largely differing results depending on the venue, and that, contrary to the general notion, laboratory experiments do not generally have greater precision than outdoor experiments (Skelly and Kiesecker, 2001; Winkler and Van Buskirk, 2012). Consequently, standard laboratory-based ecotoxicology tests may often under- or overestimate the impacts of chemical pollutants (Egea-Serrano et al., 2012; Thompson et al., 2004). One way of testing if laboratory-based experiments deliver ecologically meaningful results is to compare pesticide effects across different experimental venues (Skelly, 2002). Surprisingly, only a handful of such studies have been conducted, and most of these have only focussed on survival (Bernal et al., 2009a,b; Johnson et al., 2013; but also see Edge et al., 2013; Lanctôt et al., 2014; Saura-Mas et al., 2002).

Glyphosate-based herbicides are among the most widely applied broad-spectrum pesticides in the world (Mörtl et al., 2013; Relyea, 2005a). Glyphosate inhibits the production of essential aromatic amino acids necessary for protein synthesis and growth. Because the anionic glyphosate cannot penetrate the cuticle of many plants (Mann et al., 2009), it is usually co-administered with surfactants, such as polyethoxylated tallowamines (POEA). Glyphosate formulations with POEA and similar surfactants are for terrestrial use only, where they are considered to have very little toxicity to animals, but these herbicides can end up in aquatic habitats via spray drift, inadvertent overspray, or wash-off, and may there exert high toxicity towards the fauna at concentrations that are readily found in nature (Giesy et al., 2000; Mörtl et al., 2013; Székács and Darvas, 2012; Tsui and Chu, 2003).

As part of the global biodiversity loss, amphibians are experiencing population declines and extinctions throughout the world (e.g., Stuart et al., 2004). One of the important causes for amphibian population declines is the extensive use of pesticides (Davidson et al., 2002; Relyea, 2005b; Sparling et al., 2001). While there are taxa that are more sensitive to many chemicals (Suter, 2007), amphibians are especially vulnerable to environmental contaminants due to their thin, highly permeable skin, unshelled eggs, and complex life-cycle, exposing them to stressors both in the aquatic and the terrestrial environment. Nonetheless, amphibians have remained clearly understudied in respect to environmental contaminants (Adams and Rowland, 2003), even though results on other taxa may not be a good basis for estimating effects on amphibians during various phases of their life cycle (Linder et al., 2010; Relyea, 2004, 2003).

Glyphosate-based herbicides are moderately to highly toxic to amphibians (Lajmanovich et al., 2003; Mann and Bidwell, 1999; Relyea, 2005b). However, their toxicity is likely due to the POEA surfactant and not to the glyphosate itself (Mann and Bidwell, 1999; Perkins et al., 2000; Tsui and Chu, 2003). At sublethal concentrations, glyphosate-based, POEA-containing herbicides can cause altered development (Cauble and Wagner, 2005; Howe et al., 2004), reduced size at metamorphosis (Cauble and Wagner, 2005; Howe et al., 2004; Williams and Semlitsch, 2010), developmental malformations (Howe et al., 2004; Jayawardena et al., 2010; Lajmanovich et al., 2003), intersexuality (Howe et al., 2004), symptoms of oxidative stress (Costa et al., 2008; Güngördü, 2013) and can also affect the behaviour (Wojtaszek et al., 2004) and body shape of tadpoles (Relyea, 2012).

In this study, our aim was to evaluate the importance of the choice of experimental venue in ecotoxicology studies. To achieve this, we examined the impacts of a widely used formulation of glyphosate-based herbicide in combination with predation threat on *Rana dalmatina* tadpoles in two types of experimental venue: standard laboratory conditions vs. outdoor mesocosms. We analysed survival, development, body mass, body shape and behaviour of tadpoles exposed to one of two predator treatments (no predator, dragonfly larvae) combined with three initial herbicide concentrations (0, 2 and 6.5 mg a.e./L glyphosate) in a full factorial design. Based on previous findings, we expected to find marked differences between the two experimental settings in the effects of the herbicide on the measured life history traits (e.g., Skelly, 2002; Winkler and Van Buskirk, 2012).

2. Material and methods

2.1. Collection and maintenance of animals

We captured 30 dragonfly larvae (*Aeshna cyanea* Müller 1764) from two ponds (Bajna: 47°38′41″N, 18°36′42″E; Paprét felső: 47°44′22″N, 19°00′42″E) and transported them to the Julianna-major Experimental Station (Plant Protection Institute, Hungarian Academy of Sciences) in Budapest (47°32′52″N, 18°56′05″E). Until start of the experiment, we kept predators under laboratory conditions. We placed them individually in 300 mL cups holding 200 mL reconstituted soft water (RSW; APHA, 1985), and fed them every other day with bloodworms (*Chironomus* sp.) ad libitum. We choose this dragonfly species for predator treatments because it was not affected by the glyphosate-based herbicide formula applied by us here, according to our previous findings (Ujszegi et al., 2015). Hence in herbicide × predator treatments the herbicide presumably had no impact on the predators itself.

We collected ten freshly laid egg-clutches of the agile frog (*R. dalmatina* Bonaparte 1840) from a pond in the Pilis-Mountains, Hungary (Paprét középső: 47°44′20″N, 19°00′43″E) and transported them to Julianna-major Experimental Station. We kept clutches in 10 L containers holding 3 L RSW in the laboratory until hatching at 20 °C and a 12: 12 h light: dark cycle. Two days after hatchlings reached the free swimming state (development stage 25; Gosner, 1960) we mixed larvae from different clutches and started experiments on the same day.

2.2. Experimental setup in the laboratory

In the laboratory, we reared tadpoles individually in 2 L containers filled with 1.4 L RSW. Temperature was set to 16 °C, light