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## **Environmental Pollution**

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Life stage-specific effects of the fungicide pyrimethanil and temperature on the snail *Physella acuta* (Draparnaud, 1805) disclose the pitfalls for the aquatic risk assessment under global climate change

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#### ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form
8 October 2012
Accepted 10 October 2012

Keywords: Climate change Fungicides Toxicity tests Developmental stages Invasive species

#### ABSTRACT

It can be suggested that the combined stress of pesticide pollution and suboptimal temperature influences the sensitivity of life stages of aquatic invertebrates differently.

The embryo, juvenile, half- and full-life-cycle toxicity tests performed with the snail *Physella acuta* at different concentrations (0.06-0.5 or  $1.0~mg~L^{-1}$ ) of the model fungicide pyrimethanil at 15, 20 and 25 °C revealed, that pyrimethanil caused concentration-dependent effects at all test temperatures. Interestingly, the ecotoxicity of pyrimethanil was higher at lower (suboptimal) temperature for embryo hatching and  $F_1$  reproduction, but its ecotoxicity for juvenile growth and  $F_0$  reproduction increased with increasing temperature.

The life-stage specific temperature-dependent ecotoxicity of pyrimethanil and the high fungicide susceptibility of the invasive snail clearly demonstrate the complexity of pesticide—temperature interactions and the challenge to draw conclusions for the risk of pesticides under the impact of global climate change.

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## 1. Introduction

Increased temperature and dryness during summer months as predicted under global climate change (GCC) may induce land use changes (Bloomfield et al., 2006). An intensified use of agrochemicals is expected and additionally, predicted extreme weather events and thereby increased run-off may lead to an enhanced entry of pesticides into surface waters (Bloomfield et al., 2006; Schroll et al., 2006; Müller et al., 2010).

Fungicides represent a major class of pesticides used in agriculture (Berenzen et al., 2005). One of these fungicides is pyrimethanil which is currently used in apple orchards (application rate: 600 g ha<sup>-1</sup>) and vineyards (application rate: 1 kg ha<sup>-1</sup>) to combat mildew and gray mold. The current predicted environmental concentration of pyrimethanil in surface waters accounts to approximately 90  $\mu$ g L<sup>-1</sup> in apple orchards and 27  $\mu$ g L<sup>-1</sup> for vine cultures (EFSA, 2006). Further, the fungicide has frequently been detected in European surface waters with values up to 6.8–70  $\mu$ g pyrimethanil L<sup>-1</sup> or 272  $\mu$ g kg<sup>-1</sup> within sediments (Schlichtig

et al., 2001; Verdisson et al., 2001; Kreuger et al., 2010; Schäfer et al., 2011).

The response of aquatic communities to the combined impact of agrochemicals and GCC will strongly depend on the reaction of aquatic key species (Heugens et al., 2001; Vinebrooke et al., 2004; Thuiller, 2007; Ferreira et al., 2010; Vandenbrouck et al., 2011). Knowledge on biological reactions and adaptation potential of species and communities toward stressor combinations is however insufficient (Kwok and Leung, 2005; Lannig et al., 2006; Oetken et al., 2009). The few available studies demonstrated that the toxicity of xenobiotics most often increases under the impact of higher temperature (Cairns et al., 1975; Gagné et al., 2007). A study conducted by Schäfer et al. (2007) showed that macroinvertebrate communities can be affected at low pesticide levels due to a combined synergistic impact of multiple natural stressors and pollution in the field. Further cumulative additive effects and nonadditive interactions of natural antagonists and pollutants can result in remarkable impacts on ecologically relevant parameters (Coors and De Meester, 2005). Therefore to advance the knowledge about chronic impacts of chemicals on the aquatic environment, it is useful to expose key organisms for a longer time towards pollutants and monitor for their adaptive or sensitized responses (Bossuyt et al., 2005; Massarin et al., 2010; Staples et al., 2011;

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Salice et al., 2010; Müller et al., 2012). Our recent research on pyrimethanil supports a positive toxicity—temperature relationship. For example, *Daphnia magna* (Straus, 1820) and *Chironomus riparius* (Meigen, 1804) being generally adapted to temperatures around 20 °C reacted more sensitive to low doses of the fungicide under increased temperature (Müller et al., 2012; Seeland et al., 2012). Conversely, other studies reported a negative correlation between temperature and toxicity. For example, pesticides like DDT and pyrethroids caused a higher ecotoxicity under lower temperatures (Gordon, 2005). To further increase the complexity, the reaction to multiple stressors is often species-specific (Gordon, 2005; Deschaseaux et al., 2010).

Besides, not only the pesticide or species under investigation is of decisive importance, even the investigated developmental stage may influence the outcome of ecotoxicological temperature experiments. Sawasdee and Köhler (2009, 2010) showed that it is of utmost importance to understand the sublethal effects of toxicants on various developmental stages. This finding contrasts the majority of multiple stressor research on fish and aquatic invertebrates, where the toxicity of pesticides is mainly examined in mature aquatic stages considering mortality (Hamilton, 1995; Clearwater et al., 2002; Grosell et al., 2006; Osman et al., 2007; Vieira et al., 2009; Yadav and Trivedi, 2009). To predict the impact of temperature and xenobiotics on the development of highly sensitive growth stages, it might be useful to examine eggs (Luckenbach et al., 2001; Osterauer et al., 2009). Particularly the often pellucid eggs of snails are promising to monitor stress effects on the development of embryos (Schirling et al., 2006; Sawasdee and Köhler, 2009). Gastropods are moreover highly relevant in freshwater ecosystems and fulfill all requirements of good bioindicators (Melo et al., 2000; Oehlmann et al., 2007; Das and Khangarot, 2011).

One omnipresent, dominant and invasive mollusc is the freshwater snail *Physella acuta* (Draparnaud, 1805) (Basommatophora, Physidae) living in streams, lakes and ponds (Bacchetta et al., 2001). *P. acuta* has a holarctic origin but meanwhile it is distributed worldwide, except of the polar regions. The success of the snail in conquering new habitats is based on its high reproduction rates, high passive dispersal capacities and an advanced tolerance against disturbed environments, pollution and high

temperature (Brackenbury and Appleton, 1993; Albrecht et al., 2009). *P. acuta* is hermaphroditic and can reproduce uni- and biparentally (Sánchez-Argüello et al., 2009).

With regard to the uncomplicated monitoring of its life-stages,  $P.\ acuta$  is a very useful test organism to test the hypothesis if single and potential interactive effects of pyrimethanil and temperature differ between life stages. The hypothesis was tested by means of an embryo toxicity test, a juvenile growth test, a half-and full-life-cycle test ( $F_0$  and  $F_1$ ), which were conducted at a broad concentration range and three constant test temperatures.

#### 2. Material and methods

#### 2.1. Material

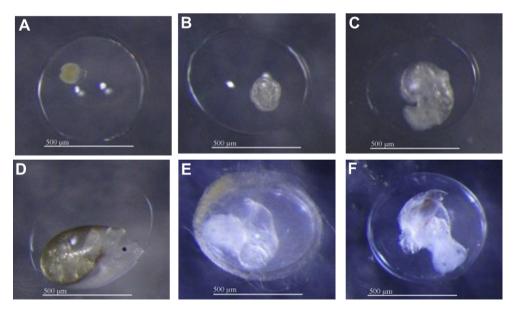
*Physella acuta* originated from our in-house culture (Institute for Ecology, Evolution and Diversity, Goethe University Frankfurt am Main). The snails were cultured in ISO medium according to the OECD guideline 202 (OECD, 2004) at a light:dark cycle of 16:8 h and 20  $\pm$  1  $^{\circ}$ C. The water was renewed once a week and snails were fed two times weekly with Tetra Min $^{\otimes}$  ad libitum.

The test substance pyrimethanil (Cas-No: 53112-28-0, PESTANAL®, analytical standard (99.9%)) was obtained from Sigma–Aldrich (Steinheim, Germany). The chemical behavior of pyrimethanil at three test temperatures was exemplarily measured in the concentration range of 0.15–2.5 mg L $^{-1}$  during six days in accordance to the HPLC protocol for water samples published by Müller et al. (2012). The nominal pyrimethanil concentrations used for ecotoxicological bioassays ranged from 0.06 to 0.5 or 1.0 mg L $^{-1}$  plus control and were received from effect concentrations determined in preliminary tests (for more information see Supplementary material).

#### 2.2. Ecotoxicological bioassays

### 2.2.1. Embryo toxicity test at three temperatures

For the embryo test, eggs were isolated from 24 h-old egg masses by removing its surrounding gelatinous mass and separated into cavities of a 24-well plate. Each cavity was filled with 2 mL of either pure or pyrimethanil-spiked ISO medium. For each treatment, twelve replicates with one egg each were exposed to 15, 20 and 25 °C in climate chambers (MKKL 1200, Flohrs Instruments GmbH, Netherlands). Once a week the water was renewed. The experiment lasted for two weeks in minimum and for four weeks in maximum, depending on the temperature-dependent full hatching success of controls. The development of the embryos was daily documented with an inverse microscope (Axiovert 40c, Zeiss, Oberkochen) by means of the following endpoints: embryonic development stage (Fig. 1), day of hatching, hatching success, mortality, and anomalies. Hatching defined as the time point when an embryo had left the egg integument, while morphologic alterations compared to the control group (malformation, cessation of development, deformation/loss of the



**Fig. 1.** Four embryonic developmental stages and deformation after exposure to pyrimethanil of *Physa acuta*. A = morula/gastrula (1st day), B = trochophora (4th day), C = veliger (6th day), D = hippo (8th day), E/F = edema and deformation of shell (0.25 mg  $L^{-1}$ ).

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