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Multiple stressor effects of herbicide, pH, and food on wetland zooplankton and a larval amphibian ☆

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Abstract

Interactions of herbicides and natural environmental stressors such as pH and food availability are poorly understood. We tested a chemical formulation of triclopyr (Release®) at environmentally relevant test concentrations (0.25 and 0.50 mg L^{-1}) in combination with two levels of pH (pH 5.5 and 7.5), and two levels of food availability (high and low). Population level effects of each stressor alone and in combination with the others were investigated using *Simocephalus vetulus*, a zooplankton species, and *Rana pipiens* tadpoles (Gosner stage 25), both common to forest ponds and wetlands. Herbicide treatments resulted in significant decreases in survival of both test species as well as reproduction and development time for *S. vetulus* at levels 5–10 × below predicted worst case environmental concentrations (2.6 mg L^{-1}). This laboratory study demonstrates a probable risk of toxic effects of Release® herbicide which may be significantly increased by low food availability and by low pH at environmentally relevant concentrations.

Keywords: Multiple stressors; pH; Zooplankton; Triclopyr; Amphibian

1. Introduction

Despite the fact that fluctuations in natural environmental conditions are known to alter effects of chemical stressors, interactive effects of stressors are not well addressed in standard toxicity test protocols (Hanazato and Dodson, 1995; Horne and Dunson, 1995; Folt et al., 1999). Combined chemical and physical stressors have been shown to create effects that are either greater than or less than those of the stressors alone. In cases where natural variation in environmental variables enhances the toxicity of chemical stressors and exacerbating conditions are likely to be common, this knowledge should be incorporated into environmental regulation and risk mitigation methods to ensure sufficient protection of non-target organisms.

This study was conducted to examine the effects of Release® in combination with two important environmental variables, pH and food availability, on wetland zooplankton and amphibians. It is part of a multiple-tiered research program for investigating the effects of herbicides used in forestry management (Thompson, 2004; Edginton et al., 2003, 2004; Chen et al., 2004; Thompson et al., 2004; Wojtaszek et al., 2004, 2005; Wojtaszek, 2004). Zooplankton and amphibians are ubiquitous in freshwater environments and known to be particularly sensitive indicators of stress (Schindler, 1987; Murphy et al., 2000). They may also be considered to be particularly vulnerable to multiple stressors as they have limited ability to migrate out of suboptimal conditions.

Herbicides are routinely used in forestry and agriculture to control the growth of unwanted plant species and increase crop production. However, in the field application

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of these chemicals, non-target habitats may be exposed to varying degree as the result of accidental overspray, drift and off-target movement (Thompson, 2004). Release® or Garlon 4®, trade names for the butoxy ethyl ester of triclopyr (TBEE), are used for the suppression of broad-leafed plants in both Canadian and US forest vegetation management regimes (Thompson and Pitt, 2003; Shepard et al., 2004). They also increase yields of spruce/fir stands and pine plantations, and are used to clear utility right-of-ways and eliminate invasive species. In surface waters, TBEE has a half-life of 3–8 days (Kreutzweiser et al., 1995; Wojtaszek et al., 2005) and is rapidly absorbed by biota, sediments and allochthonous materials (Solomon et al., 1988; Barron et al., 1990; Kreutzweiser et al., 1995; Thompson et al., 1995).

Wetlands are important habitat for vertebrates (amphibian larvae and fish), invertebrates (aquatic insects and zooplankton), and aquatic plants (algae and aquatic macrophytes) which exhibit a range of sensitivity depending on the taxon and the endpoint measured (Roshon et al., 1999). Tolerance limits for variables such as pH, food, oxygen, etc., may be narrow or wide, depending on the species. Ambient pH and food availability can vary greatly in lakes, ponds, and wetlands due to seasonal changes in temperature, productivity, and snowmelt particularly in the temperate forest regions of the US and Canada (Semkin and Jeffries, 1986; Saunders et al., 2000). For example, in wetlands and lakes of north-central Ontario, Canada, pH can range from 4.2 to 7 (Mallory et al., 1998). Both low pH and food deficiency are known to negatively affect zooplankton and amphibians resulting in lower survival, increased development times and smaller body sizes (Tessier and Consolatti, 1991; Horne and Dunson, 1995; Kupferberg, 1997; Folt et al., 1999; Locke and Sprules, 2000). Food levels below 600 μg carbon L^{-1} can be limiting to zooplankton populations if sustained over the growing season (Lampert, 1978). The combination of sub-optimal pH and food in combination with exposure to anthropogenic chemicals can result in multiple stressor effects that can diminish or enhance individual performance and population growth (Folt et al., 1999; Chen et al., 2004; Edginton et al., 2004).

In this study, we examine the individual and interactive effects of Release®, a commercial formulation of TBEE with food availability and pH. Treatment levels of herbicide, algal food, and pH are representative of conditions in natural systems. The herbicide treatments yielded initial concentrations 5–10× below predicted environmental concentrations (PEC calculated based upon maximum label rates as recommended for forestry applied directly to 15 cm of water). In these tests, exposure concentrations were held relatively constant. However, in natural surface waters the more toxic ester form of triclopyr dissipates rapidly with time to 50% dissipation ranging from <1 day up to 8 days depending on numerous factors influencing the primary dissipation mechanisms of photolysis, hydrolysis and microbial degradation (Solomon et al., 1988; Kreutzweiser et al., 1995; Wojtaszek et al., 2005).

The primary mode of action for triclopyr in plants is associated with differential cell division and growth (Ahrens, 1994) resulting in impairment of vascular transport and ultimately death of the plant. Aquatic animals, for example salmonid fry, are known to sorb TBEE from water, and then de-esterify the compound to form triclopyr acid (Barron et al., 1990). Where the de-esterification rate is less than the uptake rate, mortality may result from lethal tissue concentrations of triclopyr acid or total residues. While the exact mode of action in aquatic animals is not well understood, it has been considered to act as a general narcotic through disruption of cellular homeostasis (Barron et al., 1990; Edginton et al., 2004). Irrespective of the mode of action, toxicological literature suggests that fish, amphibians, zooplankton, plants and invertebrates are generally equi-sensitive upon exposure to formulated products containing triclopyr as the butoxyethyl ester (TBEE) (Wan et al., 1987; Johansen and Geen, 1990; Berrill et al., 1994; Roshon et al., 1999; Edginton et al., 2003; Wojtaszek, 2004). Existing triclopyr studies warrant further study of potential interactive effects on aquatic organisms.

Algal food levels reflect eutrophic to mesotrophic conditions in ponds and pH levels (5.5–7.5) span a range commonly encountered in wetlands. Specifically, we tested three hypotheses: (1) at environmentally relevant levels, Release® has no significant effects on survival, reproduction or development time of *Simocephalus vetulus* or survival of *Rana pipiens*; (2) there are no significant interactions between herbicide, low pH, and low food stressors; and (3) the two wetland taxa, *S. vetulus* and *R. pipiens*, are equally sensitive to the three stressors.

2. Methods

Zooplankton and amphibian species that have widespread geographic distributions and are representative of organisms in forest wetland environments were chosen as test organisms. *S. vetulus*, is a freshwater cladoceran species, common in shallow forest ponds. It is broadly distributed throughout subtropical and temperate regions and has been used in numerous population and toxicity studies (Willis et al., 1995). The amphibian *R. pipiens* also has a broad distribution in forest ponds and wetlands throughout North America. It is especially common in north-eastern North America where Release® is used in forestry management. Gosner stage 25 larvae were used in the experiments because of their demonstrated sensitivity to herbicide stress (Edginton et al., 2003, 2004; Chen et al., 2004; Thompson et al., 2004; Wojtaszek et al., 2004) and the coincident seasonal timing of this age class with typical forest herbicide application schedules. Both species use algae as their main food resource and we employed *Cryptomonas erosa* for both culturing and experiments. This algal species has high nutritional value (Chen and Folt, 1993) and is representative of taxonomic groups found in most lakes and ponds.

Cultures of *S. vetulus* were established from individuals collected in October 1999 from a 4 m deep, tannin-stained forest pond located in Bomoseen State Game Park in western Vermont (Coordinates: N 43.66619, W 073.22845). Zooplankton cultures were maintained at 20 °C in filtered (1.6 μm) water from Storrs Pond, NH to which EDTA (5.9×10^{-6} M Na_2EDTA) was added to enhance cryptomonad survivorship (Kirk, 1988). Storrs Pond is an eutrophic pond with pH ranging from