Interactions between atrazine and phosphorus in aquatic systems: Effects on phytoplankton and periphyton

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HIGHLIGHTS

- ▶ Atrazine has been reported to increase periphyton growth via indirect mechanisms.
- ▶ Atrazine commonly co-occurs with high nutrient runoff, which also increases growth.
- ▶ We examined effects of atrazine and phosphorus (P) on algae in a 63-d microcosm study.
- ▶ P reduced the apparent effect of atrazine on photosynthetic outputs (e.g., DO).
- ▶ No treatment-related effects on phytoplankton or periphyton biomass were observed.

GRAPHICAL ABSTRACT
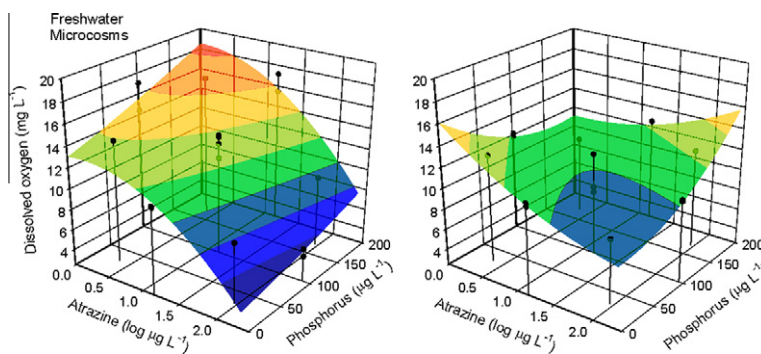
ABSTRACT

It has been proposed that the herbicide atrazine may increase rates of parasitic trematode infection in amphibians. This effect may occur indirectly as a result of increased biomass of periphyton and augmented populations of aquatic snails, which are the trematode's primary larval host. Evidence has also shown that nutrients alone may induce the same indirect responses. Since both atrazine and nutrients commonly enter surface waters from agricultural run-off, their spatial and temporal co-occurrence are highly probable. In light of recent wide-spread declines in amphibian populations, a better understanding of the role of atrazine in the proposed ecological mechanism is necessary. A microcosm study was conducted to quantify biomass of phytoplankton and periphyton over a range of atrazine and phosphorus concentrations (from 0 to 200 μg L^{-1} each) using a central composite rotatable design. Over 10 weeks, biomass and water chemistry were monitored using standard methods. Regression and canonical analyses of the response surfaces for each parameter were conducted. We found significant effects of atrazine and phosphorus on dissolved oxygen, pH, and conductivity throughout the study. Additions of phosphorus mitigated the apparent inhibition of these photosynthetic indicators caused by atrazine. Despite these changes, no consistent treatment-related differences in algal biomass were observed. These results indicate that the indirect impacts of atrazine on total growth of periphyton and likely, subsequent effects on aquatic snails, are not expected to be ecologically significant at the concentrations of atrazine tested (up to 200 μg L^{-1}) and over a range of nutrient conditions commonly occurring in agroecosystems.

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1. Introduction

The herbicide atrazine has been in use throughout the world since 1959 for the control of broadleaf and grassy weeds in agriculture. Contamination of surface waters in agricultural regions commonly occurs as a result of field run-off, and peak environmental concentrations have been found to coincide with spring-time applications (Solomon et al., 1996). In 2007, a maximum concentration of $3.91 \ \mu g \ L^{-1}$ was found in a survey of 158 locations in southern Ontario, Canada (Byer et al., 2011). In the American mid-west, higher concentrations may occur, with a maximum of $38.6 \ \mu g \ L^{-1}$ measured in the Atrazine Monitoring Program in 2011 (USEPA, 2012). Although rates of usage have declined substantially since the 1980s (McGee et al., 2010), this compound still garners significant attention from the scientific community. Due to its mode of action as a photosynthetic inhibitor, the impact of atrazine on aquatic autotrophs is a potential environmental concern. Many experimental and observational studies have addressed toxicity of atrazine to macrophytes, phytoplankton, and periphyton (an assemblage of autotrophs and non-autotrophs), and reported effect-concentrations in the literature are variable. An extensive review of the result of microcosm studies (Giddings et al., 2005) suggested that effects on aquatic plants generally are not observed below $20 \ \mu g \ L^{-1}$. Although differences due to the duration of exposure and taxa of aquatic autotrophs are commonly reported, the impact of environmental conditions on toxicity of atrazine is rarely characterized and may be an important consideration when evaluating responses under realistic conditions.

Modification of the toxicity of atrazine by light, temperature, or nutrients has been assessed for aquatic plants and algae in several recent studies. In field-grown periphyton communities, Guasch et al. (1998) found that light conditions during colonization had significant effects on toxicity of atrazine (short-term EC_{50} for carbon uptake), with greater tolerance occurring in periphyton communities grown under closed-canopy conditions compared to those under open canopies. However, variations in phosphate concentrations in the periphyton medium prior to atrazine exposure had no effect on short- or long-term toxicity of atrazine (measured by carbon uptake and chlorophyll-a, respectively) (Guasch et al., 2007). Berard et al. (1999) also found differences in toxicity of atrazine to phytoplankton based on temperature and intensity of light. Their experiments with the cyanobacteria *Oscillatoria limnetica* in laboratory culture showed increasing sensitivity to atrazine with declining temperature (13 °C vs. 20 °C). In their mesocosm experiments with complete phytoplankton communities, growth of *O. limnetica* was suppressed during the cooler, darker spring season (<15 °C), and stimulated during the warmer, brighter summer (>15 °C) at $10 \ \mu g \ L^{-1}$ atrazine.

Changes in environmental conditions such as nutrient and light levels may also be induced indirectly by the contaminant itself. A 2008 mesocosm study by Rohr et al. (2008) proposed that a reported reduction of phytoplankton growth as a result of atrazine exposure ($117 \ \mu g \ L^{-1}$) caused an increase in light and availability of nutrients, resulting in increased growth of the periphyton community in atrazine-treated mesocosms compared to controls. As snails are grazers of periphyton, populations of snails in these test systems were observed to increase under atrazine treatment, along with populations of plagiorchid trematodes, which use the snail as a primary intermediate host. Certain snail-hosted trematodes (*Riberoria* spp.) can cause limb deformities in adult frogs, and *Echinostoma* spp. which cause edema in tadpoles (Fried et al., 1997) may also be of concern in regions of atrazine usage (Koprivnikar et al., 2007).

In 2009, we conducted an outdoor microcosm study (Baxter et al., 2011; Baxter et al., 2012) to determine whether this proposed mechanism exhibits a concentration–response relationship, since $117 \ \mu g \ L^{-1}$ is two orders of magnitude higher than commonly observed in surface waters of Ontario and the American midwest (Giddings et al., 2005; Byer et al., 2011). We tested atrazine concentrations of 0, 1, 10, 30, and $100 \ \mu g \ L^{-1}$, and observed no consistent effects on phytoplankton, periphyton or snail populations. However, ambient nutrient concentrations in our microcosms were in the eutrophic range (Kalff, 2001) (0.45–$1.8 \ mg \ L^{-1}$ total phosphorus), and the pathway proposed by Rohr et al. (2008) may only occur under nutrient-limited conditions, as reductions in phytoplankton are thought to produce a boost in available nutrients for periphyton. In another recent study, nutrients alone have been shown to play a significant role in loadings of amphibian parasites as a result of increased periphytic growth. Johnson et al. (2007) observed increased growth of periphyton along with increasing populations of snails and trematodes under nutrient-enriched conditions in the absence of atrazine, indicating that initial concentrations of nutrients may be as, or more important than atrazine in mediating these responses. Furthermore, in a field study associated with the mesocosm work by Rohr et al. (2008), phosphate at approximately 20–$630 \ \mu g \ L^{-1}$ was the second most important variable that correlated with rates of parasite infections in wetland frogs, after atrazine at concentrations of approximately 0.04–$0.58 \ \mu g \ L^{-1}$. Since inputs of atrazine and nutrients both commonly occur in agricultural run-off (Solomon et al., 1996), an investigation into possible interactions was judged necessary to better understand this process.

The objectives of this study were to determine the combined effects of phosphorus (as the common limiting nutrient for plant growth in freshwater systems) and atrazine on the biomass of phytoplankton and periphyton under semi-field conditions. Outdoor microcosms were used to replicate natural freshwater ponds and a central composite rotatable design was chosen in order to maximize statistical power based on the limited availability of experimental units (microcosms).

2. Materials and methods

2.1. Microcosms

This study was conducted over 63 d (July 6 to September 7, 2010) at the University of Guelph Turfgrass Institute Microcosm Facility (Guelph, ON). A detailed description of the microcosms can be found in Hanson et al. (2005). Since exchange of phosphate occurs at the sediment interface (Bostrom et al., 1988), no sediment was used in the microcosms to better manage concentrations of nutrients in the water column. The microcosms were filled with well-water from the adjacent irrigation pond and circulated at a rate of $12\,000 \ L \ d^{-1}$ for three weeks to establish uniformity in biological and physico-chemical properties. Circulation ceased 2 d prior to treatment to create isolated and independent experimental units.

In addition to the analyses described below, it was our intention to assess the response of snail populations during the study. We relied on natural colonization of the microcosms during the circulation-phase, as previously described (Baxter et al., 2011), but colonization was too low for inclusion in the analysis (no snails were observed in 64% of sampling events). Although this limits the discussion of results to physico-chemical and algal responses to treatment, it is valuable to fully understand this first link in the proposed chain of effects.

2.2. Study design and statistical analysis

In order to accommodate the two-factor design with a limited number of ponds available, we used a central composite rotatable