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EFFECTS OF ATRAZINE ON AMPHIBIAN GROWTH AND SURVIVAL IN ARTIFICIAL AQUATIC COMMUNITIES

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Abstract—Artificial pond microcosms with pond water, including phytoplankton, periphyton, macrophytes, and larval gray tree frogs (*Hyla versicolor*), were treated with atrazine to achieve final aqueous concentrations of 0, 20, 200, or 2,000 μg/L. Dissolved oxygen concentrations (DO) decreased to approx. 20 to 40% of their preexposure values in the 200- and 2,000-μg/L treatment groups within 1 d of atrazine addition. Dissolved oxygen in these microcosms returned to control concentrations by 10 d after exposure but declined again to approximately 60 to 80% of control values at 21 d after exposure and remained depressed for the remainder of the study. In the 200- and 2,000-μg/L treatment groups, pH decreased similarly within 1 d of atrazine exposure and returned to control values within the following 16 d. The DO and pH did not differ significantly between the 0- and 20-μg/L groups or the 200- and 2,000-μg/L groups. Frogs from the two higher atrazine concentration treatment groups were 5% shorter and had 10% lower body mass at metamorphosis than those from the control and low atrazine concentration groups. No difference in length or body mass at metamorphosis was detectable between the 0- and 20-μg/L groups or between the 200- and 2,000-μg/L groups. Larval period was 5% longer in the 2,000-μg/L group than in the 200-μg/L group but did not differ from controls in any treatment group. No significant treatment-related differences were detected for survival rate. The decrease in amphibian length and weight at metamorphosis may indicate a reduction in fitness in wild populations of anurans exposed to atrazine at 200 to 2,000 μg/L.

Keywords—Atrazine Herbicide Amphibian Frog Dissolved oxygen

INTRODUCTION

A mounting body of evidence suggests that many amphibian species, across a wide spectrum of habitat types, have experienced substantial declines in number and distribution. Although contributory factors have been suggested in declines of several species [1–8], no unifying cause has been identified. The ultimate causes of these declines are likely numerous and varied. Amphibians in the midwestern United States face an environment profoundly different from that which existed before European settlement. It has been estimated that more than 90% of the natural wetlands in Illinois have been lost [9]. Many of the remaining natural or constructed wetlands are heavily affected by human development and agriculture. Considering the magnitude of acreage involved, as well as the intensity and character of management, midwestern agriculture represents an enormous release of chemicals to the environment. Thus, to identify causative factors of regional amphibian population declines, it is important to assess potential adverse effects of agrochemicals.

Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-*s*-triazine) is used widely for weed control in corn and sorghum production. Usage of atrazine in the United States in 1993 was estimated at greater than 32,000 tons, more than 50% of which was applied in Illinois, Iowa, Nebraska, and Indiana [10].

Atrazine is an inhibitor of photosynthesis and is far more toxic to plants than animals. Most fish, amphibians, zooplankton, and benthos are not acutely poisoned by atrazine at concentrations found in the environment [11,12]. Macrophytes and phytoplankton, however, are adversely affected at mean concentrations of approx. 100 μg/L, and individual phytoplankton species may be sensitive at much lower concentrations [13,14]. It has been suggested that 20 μg/L should be regarded as a no-observed-effect level for atrazine in surface waters [11,15].

Atrazine in naturally occurring freshwater has been reported to be relatively persistent. Values for atrazine half-life in freshwater mesocosms or pond ecosystems have been reported in, or calculated from, the literature at 170 to 350 d [16], 260 d [17], 230 d [18], 95 d [18], and 8 to 14 d [12], the latter in a flow-through wetland mesocosm with highly organic sediments.

Herbicides such as atrazine at concentrations known to be directly toxic only to plants have been reported to affect animals in complex communities when an additional stressor was added to the system [16,17,19]. Although many community-based toxicology studies have been performed to identify direct and indirect effects of specific toxicants, few have involved amphibians. A decrease in biomass of larval *Rana catesbeiana* was noted in ponds treated with commercial or reagent-grade atrazine at concentrations as low as 20 μg/L when grass carp were also present [16]. Because this is well below concentrations known to cause toxic effects in vertebrates, the effect was assumed to be indirect, secondary to a decrease in macrophyte, phytoplankton, or periphyton biomass within the treated ponds. The amphibians inhabiting the ponds were volunteers, so it was not possible to differentiate between an indirect effect of the toxicant on growth and survival of larvae or a decrease in the desirability of treated ponds as breeding sites [16].

Larval anurans feed on phytoplankton and periphyton [19–21], and exposure of aquatic communities to atrazine has been

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shown to decrease phytoplankton and periphyton biomass [17]. Recovery of phytoplankton and periphyton biomass after atrazine exposure typically results from proliferation of atrazine-resistant species [17,22]. Food availability often is a limiting factor for larval anurans [21,23]. Thus, exposure of aquatic communities to atrazine likely increases competition for the remaining food, forces larval anurans to rely on potentially less desirable food sources, and slows growth.

A decreased rate of larval growth increases the time that larvae are within acceptable size ranges for small predators, such as salamanders and predatory insects [24–26]. The effects of predation in regulating larval anuran populations are substantial. Growth rate, therefore, because of the effect of predation, likely has a profound effect on amphibian fitness, population size, and stability. Additionally, simplification of the physical habitat increases susceptibility of larval anurans to fish predation [27,28].

A decreased rate of growth also extends the larval period, delaying emergence from the water, and/or decreases the size of animals at metamorphosis. Both delayed emergence and small size at metamorphosis have been associated with a delay in the first reproductive attempt, a greater risk of mortality before breeding is attempted, potentially fewer breeding attempts over the life of the animal, and decreased egg clutch size [29–32].

Potential indirect effects on larval amphibians also may arise from atrazine-induced inhibition of community photosynthesis and resultant reductions in dissolved oxygen (DO) [16,17]. Larval anurans in water with low DO (45–50 Torr at 25°C, or approx. 1.8 mg/L) increased respiratory effort at the expense of feeding when they were not allowed to take air from the surface of the water [32]. Although larvae of several species of anurans, including gray tree frogs (*Hyla versicolor*), are known to lung-breathe when DO is low, this ability is not operative immediately on hatching; thus, young larvae may be particularly vulnerable to low DO.

The goal of this study was to determine the effects of atrazine on survival, mass and length at metamorphosis, and days to metamorphosis of larval gray tree frogs (*Hyla versicolor*) inhabiting artificial pond microcosms and to assess interrelationships among these parameters and DO concentrations, water pH values, and estimates of phytoplankton, periphyton, and macrophyte biomass.

MATERIALS AND METHODS

Microcosm design

In mid-May 1996, artificial pond microcosms were constructed outdoors at the experimental pond facility of the Illinois Natural History Survey in Urbana, Illinois, USA, using a modification of the models of Morin [33] and Van Buskirk [34]. Sixteen plastic wading pools (1.22-m diameter) were arranged in a 4×4 array on a gravel pad and were filled with 90 L of water from a nearby pond. Dried wheat straw (150 g) was added to each microcosm to provide slow nutrient release. Eighty stems of the aquatic macrophyte *Polygonum hydropiper* were collected from a second pond, potted, and added five to a pool. Unscreened glass microscope slides were suspended in the water of each pool for weekly quantification of chlorophyll *a* in the adherent periphyton. The microcosms were covered with 3.2 mm mesh fiberglass screen to exclude predators and to contain the added amphibians. Clear 6-μm-thick greenhouse polyethylene film (703, CT Film, Harrington, DE, USA) covers were suspended 43 cm above the microcosms to prevent uncontrolled additions of rainwater and dilution of atrazine. Each cover protected two microcosms. The microcosms were allowed to stabilize for five weeks before atrazine addition. Water lost to evaporation was replaced with deionized water twice weekly.

An amplectant *H. versicolor* pair was captured and oviposited in captivity on June 5, 1996. The resulting larvae were held indoors in pond water and fed Nasco frog brittle (Fort Atkinson, WI, USA) until their addition to the microcosms. On June 14, 1996, 40 larvae were added nonselectively to each microcosm.

An aqueous solution of technical grade atrazine (Ciba-Geigy, Greensboro, NC, USA) was produced by first preparing a solution of atrazine in acetone, at 5.002 mg/ml, adding this solution dropwise with constant stirring to deionized water to a final concentrations of 30.01 mg/L. Air was bubbled through the solution overnight in order to volatilize the acetone from the aqueous solution. Acetone was added to deionized water as a vehicle control solution, and air was bubbled through this overnight as well. Each microcosm was treated with 6 L of a combination of aqueous atrazine solution and/or vehicle to achieve final atrazine concentrations of 0, 20, 200, or 2,000 μg/L with four replicates of each treatment and control group. Atrazine concentrations evaluated in the current study included 20 μg/L, the no-observed-effect level as interpreted by Solomon et al. [11], as well as 200 μg/L, a concentration found in static water bodies that receive runoff from atrazine-treated farm fields [35], and 2,000 μg/L, a highly phytotoxic concentration that was considered unlikely to cause acute toxic effects in tadpoles. The solutions were sprinkled evenly over the surface of each microcosm using a watering can with a fine-spray head. The water in each microcosm was mixed briefly following treatment using a strip of clean wood. The treatments were arranged within the array of 16 microcosms in a Latin square design.

Amphibian assessment

Snout-vent length, body mass, and date of metamorphosis were determined for all frogs that completed metamorphosis within each pool. Metamorphs were collected from microcosms every night as soon as they were seen clinging to the sides of the pools and were held in a laboratory at 26.7 ± 1°C until tail resorption. The animals were then killed by immersion in 3-amino benzoic acid ethyl ester (MS-222) at 1:1,000 in water. The date of completion of metamorphosis was recorded, and body mass was determined to the nearest 0.001 g (Sartorius model 1712 MP8, Gottingen, Germany). Snout-vent length was measured to the nearest 0.05 mm. Survivorship was calculated from a count of animals that completed metamorphosis after searching each microcosm to ensure that no *H. versicolor* larvae remained.

Microcosm monitoring

For atrazine analysis, water samples initially were collected from the pond that served as the water source and then from each microcosm immediately after, and again three weeks after, atrazine addition. Atrazine analyses were performed using the Ohmicron Atrazine RaPID Assay (Ohmicron Environmental Diagnostics, Newtown, PA, USA). The intra-assay and inter-assay coefficients of variance were 3.91% and 1.24%, respectively.

Water samples of 1 L each were collected from each microcosm for quantification of chlorophyll *a*, as an indicator of

phytoplankton biomass, before the addition of atrazine and weekly throughout the study. Two of the suspended glass microscope slides were removed weekly from each microcosm for quantification of chlorophyll *a* in the adherent periphyton. Chlorophyll *a* concentrations were determined by acetone extraction and measurement of absorbance at 630, 647, 664, and 740 nm using a split-beam spectrophotometer (Lambda 3, Perkin-Elmer, Oak Brook, IL, USA) with quartz cuvettes (1-cm path length) according to the U.S. Environmental Protection Agency Method 446.0 for chlorophyll *a* analysis [36]. The intraassay and interassay coefficients of variation were 1.2% and 1.58%, respectively, and recovery efficiency was 89.29 ± 2.77%.

Unlike all other microcosms, one negative control microcosm had an early and sustained bloom of zooplankton, causing it to clear of phytoplankton early and remain clear. Consequently, this microcosm was removed from consideration during data analysis.

Macrophyte biomass was determined after the microcosms were drained at the end of the study. All macrophytes were harvested at the soil line, blotted dry, and immediately weighed (TL 1600G, American Scientific Products, San Diego, CA, USA).

The DO, temperature, and pH were measured in each microcosm before dawn and before dusk daily from 1 d before until 10 d after atrazine treatment and every 3 d thereafter until 51 d after atrazine or vehicle addition. Temperature and DO were measured using a YSI model 57 oxygen meter (Yellow Springs, OH, USA), and pH was determined using a Corning Check-Mite portable pH meter (Corning, NY, USA).

Data analysis

Data were analyzed using the SYSTAT 7.0® software package (SYSTAT, Chicago, IL, USA). Because of the possible effect of pool position on temperature, morning and evening temperatures were analyzed separately by repeated-measures analysis of variance with column and row as independent variables in two separate analyses. Larval period duration also was analyzed by life-table analysis.

The proportion of the larvae surviving through metamorphosis was transformed into the arcsine of the value to obtain a normal distribution of values. This transformed value, as well as untransformed values for macrophyte biomass, were subjected to a one-way analysis of variance with atrazine concentration as the independent variable. When an overall difference among treatment groups was detected with the analysis of variance, treatment means were compared using the Tukey HSD multiple comparison test and linear contrasts.

Phytoplankton chlorophyll *a* concentrations, DO concentrations, and pH values were analyzed by repeated-measures analysis of variance, with atrazine concentration, column, and row as independent variables. For all the previously mentioned analyses, a value of $\alpha = 0.05$ was chosen to detect significant differences. Least-significant differences were calculated for repeated-measures analyses, using a 95% confidence level, for comparisons of daily treatment means at the same times as well as of means between two times for a given treatment group. Chlorophyll *a* concentrations from periphyton collected from glass substrates were almost uniformly below the limit of detection of the assay and were not included in the data analysis.

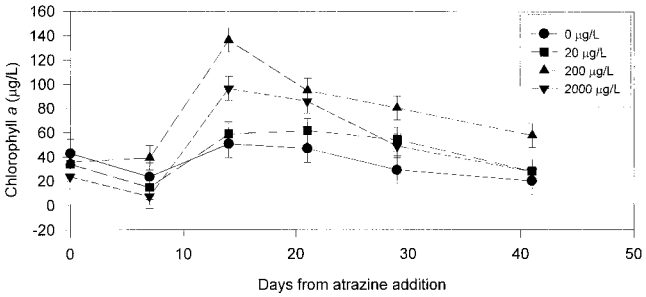

Fig. 1. Mean phytoplankton chlorophyll *a* concentrations (± standard error of mean [SE]) as a function of atrazine concentrations and time. $N = 3$ for the control group and 4 for each atrazine-treated group.

RESULTS

The concentrations of atrazine detected in microcosms immediately following atrazine addition were consistent with those intended and showed minimal variation within treatment groups (mean ± SE for 0-, 20-, 200-, and 2,000-µg/L treatment groups were 1.64 ± 0.09, 20.13 ± 0.58, 195.50 ± 8.74, and 2,035.83 ± 51.54 µg/L, respectively). By three weeks after addition of atrazine, herbicide concentrations had declined by 21%, 9%, and 16% in the 20-, 200-, and 2,000-µg/L treatment groups, respectively.

Effects on plants

Phytoplankton chlorophyll *a* concentrations (Fig. 1) changed significantly over time within treatment groups ($p < 0.001$) and differed significantly among treatment groups overall ($p = 0.01$). This parameter declined only slightly, and insignificantly, during the first week following atrazine addition in all but the 200-µg/L treatment group. By day 14, phytoplankton populations in atrazine-treated mesocosms had rebounded to densities well above those present before exposure and, with the exception of the 20-µg/L treatment group, well above those of controls. No significant differences in phytoplankton chlorophyll *a* concentration were detected between control and 20-µg/L treatment groups at any time. Phytoplankton densities in the 200- and 2,000-µg/L treatment groups were not significantly different at any time. Phytoplankton densities in the 200- and 2,000-µg/L treatment groups, however, increased significantly above controls during the rebound period, and the maximal phytoplankton density occurred in the 200-µg/L group. Over the course of the study, phytoplankton chlorophyll *a* concentration was lowest in controls, highest at 200 µg/L, and intermediate in the 20- and 2,000-µg/L groups.

Macrophyte biomass at the end of this study was decreased, relative to controls, by 30%, 98%, and 99% in microcosms containing atrazine in the 20-, 200-, and 2,000-µg/L groups, respectively (Fig. 2). The overall effect of atrazine concentration on macrophyte biomass was highly significant ($p < 0.001$). As expected, macrophytes were killed at the two highest concentrations, and the difference in macrophyte biomass between these treatment groups was not significant. Macrophyte biomass was greater in controls than at 20 µg/L, but the difference was not significant ($p = 0.104$).

Effects on water chemistry

Predusk DO (Fig. 3a) decreased markedly and almost immediately in the 200- and 2,000-µg/L dose groups, with a recovery to control values by day 10. Interestingly, these two groups again deviated from the control and 20-µg/L groups

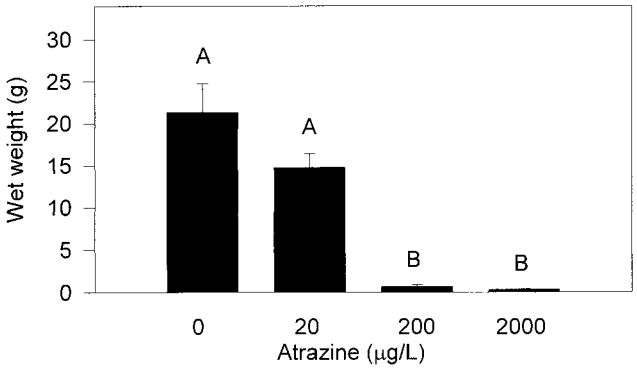

Fig. 2. Mean macrophyte wet weight (± standard error of mean [SE]) as a function of atrazine concentrations. Different letters indicate that groups differed significantly ($p < 0.05$). $N = 3$ for the control group and 4 for each atrazine-treated group.

after day 20. Atrazine exposure was associated with significant differences among treatment groups in overall predusk DO as well as in the change of predusk DO over time ($p < 0.001$ in both cases). Atrazine also was associated with a transient decline in predawn DO concentrations (Fig. 3b). Predawn DO changed significantly over time in all treatment groups ($p < 0.001$), and atrazine exposure was associated with significant differences among treatment groups in overall predawn DO ($p = 0.007$) as well as in the change in predawn DO over time ($p = 0.002$). Nevertheless, the atrazine-induced reduction in predawn DO concentration was small even at the higher concentrations, on the order of 0.5 mg/L over the course of the study.

Morning and evening pH changed significantly with time over all treatment groups and differed among treatment groups overall ($p < 0.001$ in each case) (Fig. 4a and b). Despite the marked decrease in pH associated with atrazine, pH values generally remained basic. Even in microcosms containing atrazine at 2,000 μg/L, pH declined to a minimum of approx. 7.2. In untreated microcosms, or those with atrazine at 20 μg/L, pH routinely increased by the end of each measurement day, commonly to above 9.

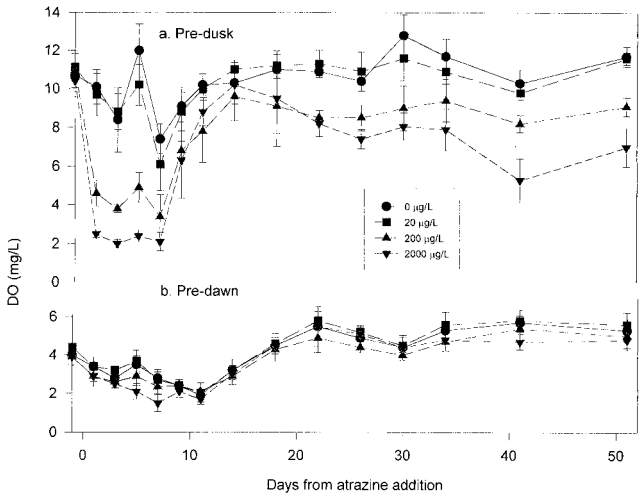

Fig. 3. Mean (± standard error of mean [SE]) (**a**) Predusk and (**b**) predawn dissolved oxygen (DO) concentrations as a function of atrazine concentrations and time. $N = 3$ for the control group and 4 for each atrazine-treated group.

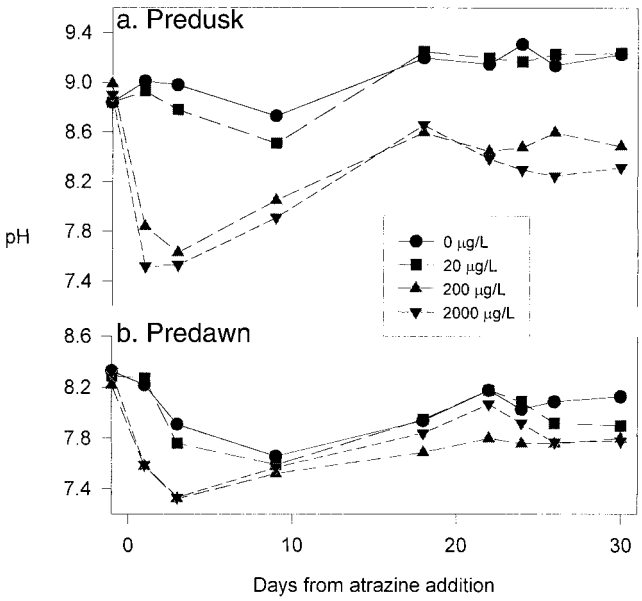

Fig. 4. Mean (**a**) predusk and (**b**) predawn pH as a function of atrazine concentrations and time. $N = 3$ for the control group and 4 for each atrazine-treated group.

Effects on amphibians

At metamorphosis, frogs in pools exposed to atrazine at 200 or 2,000 μg/L were 5% shorter and had 10% lower body mass ($p < 0.001$ in each case) than those in microcosms exposed to atrazine at 0 or 20 μg/L (Table 1). Larval period duration was 5% longer in the 2,000-μg/L group than in the 200-μg/L group ($p = 0.025$) but did not differ significantly among any other groups (Table 1). By contrast, no significant effect of atrazine exposure on larval period duration was detected using life-table analysis (data not shown). Although percentage survival to metamorphosis was lowest in the 2,000-μg/L treatment groups, the difference was not significant from the other treatment groups (Table 1).

Length and mass at metamorphosis varied with time after exposure and thus with larval age (Fig. 5). Snout-vent length and mass of animals that emerged from the pools decreased from week 1 following atrazine exposure to week 2, then gradually increased. In the control and low-dose microcosms, the increase in length and mass continued until all animals had emerged. In the mid- and high-dose microcosms, however, length and mass decreased during the final week that animals emerged.

DISCUSSION

Given the lack of a profound and prolonged decrease in phytoplankton chlorophyll *a* concentration and the magnitude of the subsequent compensatory growth of phytoplankton, it seems unlikely that the effects of the herbicide on amphibian development were due to an absolute decrease in the availability of phytoplankton or periphyton as food. However, the possibility remains that the atrazine-resistant species that occurred in the presence of continued exposure to the herbicide may be less palatable, of lower nutritive value, or toxigenic.

The more substantial rebound in phytoplankton biomass, and the greater phytoplankton biomass over the course of the study in the mid- and high-concentration groups, was likely due to the elimination of macrophytes. The surviving macrophytes presumably competed with phytoplankton for nutri-

Table 1. Survival through metamorphosis, larval period duration (d), mass (g), and length (mm) at metamorphosis as a function of atrazine concentrations. The total *N* for the treatment concentrations 0, 20, 200, and 2,000 μg/L were 97, 134, 135, and 112, respectively

Atrazine concentration (μg/L)	No. of replicates	Percentage survival[a]	Larval period duration	Mass at metamorphosis	Length at metamorphosis
0	3	80.8 ± 5.6[b]	13.38 ± 0.59	0.482 ± 0.026	19.00 ± 0.28
20	4	83.1 ± 7.9	13.55 ± 0.59	0.484 ± 0.021	18.9 ± 0.20
200	4	84.4 ± 4.1	12.39 ± 0.76	0.449 ± 0.019[c]	18.0 ± 0.20[c]
2,000	4	70.7 ± 4.9	13.99 ± 0.69[d]	0.448 ± 0.021[c]	18.1 ± 0.20[c]

[a] Differences were not significant at $p < 0.05$.
[b] Data are expressed as mean ± standard error of mean (SE).
[c] Significantly less than the 0- and 20-μg/L treatment groups ($p < 0.001$).
[d] Significantly greater than the 200-μg/L treatment group ($p = 0.025$).

ents and may have inhibited phytoplankton growth by allelopathy. The low phytoplankton biomass in the highest-concentration group may represent growth inhibition by atrazine even of the resistant organisms that proliferated in the herbicide-treated microcosms. An atrazine-induced transient decrease in phytoplankton followed by a subsequent rebound and bloom of phytoplankton as resistant species exploit the resource-rich habitat created by both plant decay and reduced assimilation of inorganic nutrients by surviving plants has been noted previously [17]. Even though in the current study chlorophyll *a* concentrations for the 2,000-μg/L group approached zero after 7 d of exposure, this initial decline was not statistically significant. The failure to detect a significant initial decline in phytoplankton biomass in this experiment may have been due to a low initial phytoplankton biomass as well as variability within treatment groups both before and immediately after atrazine exposure.

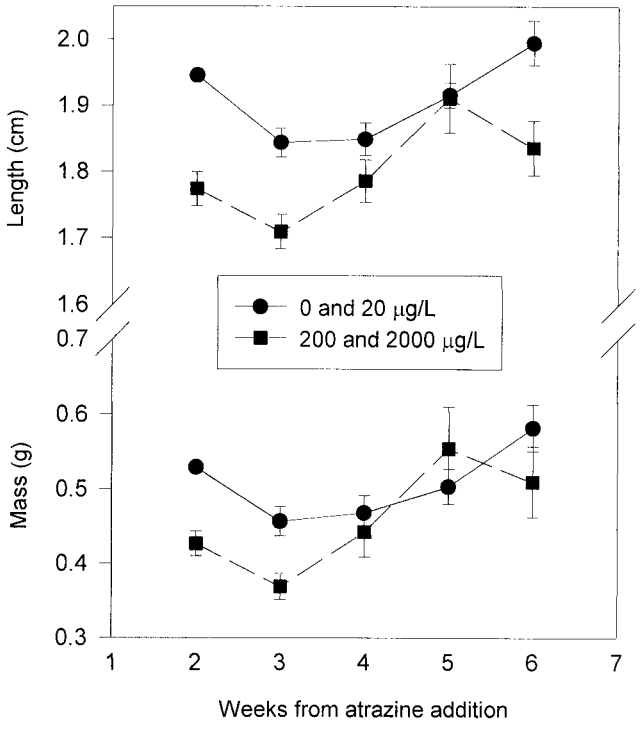

Fig. 5. Mean (± standard error of mean [SE]) snout-vent length and mass at metamorphosis as a function of atrazine concentrations and time. Statistically indistinguishable groups are combined. At two weeks, animals had emerged from only one microcosm in the upper curve; therefore, no SE is presented for this point. $N = 7$ microcosms for the pooled 0- and 20-μg/L groups and 8 for the pooled 200- and 2,000-μg/L groups.

Given the modest decline in phytoplankton biomass and the marked effects of atrazine on DO, it appears likely that the adverse effects of atrazine on amphibian growth are mediated primarily by decreased oxygen availability. Transient declines in DO due to atrazine have been reported previously; however, the secondary decline of DO, beginning 20 d following atrazine addition in the two higher-concentration groups, has not. It is possible that this effect was due to elimination of macrophytes in the mid- and high-concentration groups, rather than inhibition of phytoplankton and periphyton photosynthesis, because phytoplankton density by day 20 was lowest in controls and highest at 200 μg/L. Although the initial decreases in predusk DO were relatively short-lived, they were severe and may have placed a significant stress on the experimental animals.

The larvae used in the present study were 15 d old and 11 d posthatch at the time that atrazine was added to the microcosms. Larvae of many anuran species, including *H. versicolor,* are able to breathe air at a very young age. Feder et al. [37] showed that larval *X. laevis* increased their effort at gill respiration, at the expense of feeding, when held in water with a low oxygen content. This effect was eliminated, however, when access to air was allowed. While the larvae used in the present study were likely impacted by the severely decreased DO in the 200- and 2,000-μg/L treatment microcosms, younger larvae, which had not yet developed functional lungs, would likely experience more severe effects.

Despite greater overall larval mortality in the 2,000-μg/L treatment group (Table 1), there were no significant differences among groups associated with atrazine exposure ($p = 0.486$). However, it is conceivable that, at the high dose of technical grade atrazine used in the present study (2,000 μg/L), some larvae were killed by the herbicide directly. The 2,000-μg/L concentration is within the range reported to be acutely lethal to the larvae to other life stages of amphibians. Birge et al. [38], for example, reported 96-h LC50s for atrazine ranging from 410 μg/L in embryonic *R. catesbeiana* to 48,000 μg/L in embryonic *Bufo americanus.* In that study, however, the formulation of atrazine used was not reported, raising the possibility that the reported effects were due in part to compounds other than atrazine. By contrast, the current investigation employed technical grade atrazine rather than a commercial herbicide product. This study was not designed to distinguish between direct and indirect effects of atrazine on amphibians. The exposures in this study were chronic and involved an anuran species not examined previously in this context. Direct

toxic effects, therefore, cannot be ruled out simply on the basis of the concentrations of atrazine used.

The relationships of metamorph length and weight with time were curvilinear and were affected by atrazine exposure. Because the initial decline in length and weight was seen in all treatment groups, it appears likely that such reductions are normal for *H. versicolor* in the environment provided. The decline in length and weight of late metamorphs from mid- and high-concentration microcosms, however, appears to be related to atrazine exposure. The differences in snout-vent length and mass at metamorphosis identified previously were relatively small considering the high concentrations of atrazine used in the study. Nevertheless, the observed effects of atrazine on growth, development, and survival of larval amphibians may substantially affect survival under field conditions for several reasons. First, even the small experimental effect identified in this study may represent a substantially increased risk of predation during the larval phase in a more complex and realistic community. Maturing larvae experience reductions in both mass and length as they approach metamorphosis and profound differences in larval size become less apparent as the animals mature. For example, an 11% decrease in weight at metamorphosis was identified in barking tree frogs (*Hyla gratiosa*) raised under transient nutrient restriction when compared to control groups fed ad libitum [39]. However, when the tadpoles were weighed approximately halfway through larval development (day 41), those on the restricted diet weighed 38% less than controls. In addition, Caldwell et al. [24] exposed larval barking tree frogs to larval dragonflies (*Anax junius*) and reported a 95% higher 24-h predation rate for larvae in the 30- to 39-mm size class compared to those in the 40- to 49-mm size class. In the current study, weight and length were not determined on experimental animals until metamorphosis was completed, so a similar size and weight disparity of larvae cannot be established. However, it is likely that the 5% reduction in length and the 10% reduction in body mass at metamorphosis identified in the current study may represent far more profound differences in size and thus in susceptibility to predation during larval development.

CONCLUSIONS

This study does not contradict the assertion by Solomon et al. [11] and Huber [15] that 20 μg/L is the no-observed-effect level for atrazine in aquatic communities. By contrast, adverse effects were seen in microcosms exposed to atrazine at 200 and 2,000 μg/L. Therefore, the no-observed-adverse-effect level for atrazine in the experimental system used in this study is between 20 and 200 μg/L. Based on the observed decreases in length and mass at metamorphosis as well as decreases in pH, DO, and macrophyte biomass, it is suggested that these would tend to set the stage for increased risks of predation as well as decreased fitness. Relatively simple communities were used in the present study in order to evaluate the effects of atrazine contamination on specific end points indicative of subsequent fitness of the experimental animals. Future studies involving amphibians of differing ages and species, different end points, and more complex model communities inclusive of predators, herbivores, parasites, and combinations of atrazine with other pesticides and/or fertilizers should serve to refine or revise this estimate. In addition, field studies will be required to understand the full impact of atrazine on amphibians in the wild.

The upper Midwest has experienced marked declines in amphibians. For example, Lannoo et al. [40] estimated that amphibian numbers have declined by three orders of magnitude in northwestern Iowa since the early 1900s. This entire area is dominated by production of corn, and by far the most widely used herbicide in these fields is atrazine [10]. Because of its potent activity as an herbicide, the plant community in these areas may experience intermittent harmful effects, and these are likely to cause secondary effects on the amphibian community, including *H. versicolor*. The reduced fitness in this and other amphibian species that may follow atrazine exposure of local ecosystems is likely to set the stage for increased losses due to reduced rates of growth and increased predation.

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