JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION
Vol. 48, No. 3 AMERICAN WATER RESOURCES ASSOCIATION June 2012

EXPOSURE TIMES TO THE SPRING ATRAZINE FLUSH ALONG A STREAM-RESERVOIR SYSTEM[1]

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ABSTRACT: We used enzyme-linked immunosorbent assay to examine reservoir-mediated shifts in spring to fall exposure of aquatic organisms to the spring atrazine pulse over four years in a Midwestern stream-reservoir system. Peak atrazine concentrations in the major inflowing stream exceeded 10 μg/l in all four years. The reservoir had a beneficial effect in two of four years by diluting atrazine below the 10 μg/l threshold. However, during the other two years, exposure times above 10 μg/l were approximately doubled in the reservoir compared to the major inflowing stream. Thresholds of 3 and 5 μg/l were exceeded during all four years in the reservoir. The uplake and downlake reservoir sites were four to five times more likely to exceed these thresholds and aquatic organisms were subjected to longer exposure times above these thresholds compared to the inflowing stream. Release of elevated atrazine concentrations from the reservoir extended exposure times in the outflowing stream. This effect was most pronounced just below the dam. Aquatic organisms upstream of the reservoir were most likely to experience acute exposures whereas organisms within and immediately downstream of the reservoir were more likely to experience chronic exposures. The ubiquity of reservoirs and the annual spring herbicide flush highlight the importance of considering the presence and relative location of reservoirs when assessing risk to aquatic communities as well as locations of drinking water intakes.

(KEY TERMS: atrazine; reservoir; retention; exposure; spring flush; herbicide.)

Stoeckel, James A., Jade Morris, Elizabeth Ames, David C. Glover, Michael J. Vanni, William Renwick, and María J. González, 2012. Exposure Times to the Spring Atrazine Flush Along a Stream-Reservoir System. *Journal of the American Water Resources Association* (JAWRA) 48(3): 616-634. DOI: 10.1111/j.1752-1688.2011.00633.x

INTRODUCTION

Herbicides are now ubiquitous in North American waterways. Even pristine areas are exposed to herbicides and pesticides via atmospheric deposition (Thurman and Cromwell, 2000; Mast *et al.*, 2006). Waterbodies in agriculturally dominated watersheds may receive particularly large inputs of herbicides from farm field runoff following periods of heavy rain (Thurman *et al.*, 1991, 1992). Reservoirs are susceptible to inputs of agricultural chemicals due to their

[1]Paper No. JAWRA-11-0018-P of the *Journal of the American Water Resources Association* (JAWRA). Received February 10, 2011; accepted December 12, 2011.

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strong linkage to connected lotic systems whose flow and chemical load are greatly increased by storm events. Following a period of heavy rain, reservoirs can act as storage units for herbicides. Throughout much of the United States (U.S.), highest annual inflow to reservoirs occurs during the spring/early summer season (Thurman *et al.*, 1991; Solomon *et al.*, 1996) coinciding with the seasonal period of highest herbicide usage, increasing the likelihood of herbicide flush into streams and rivers from agricultural fields (Thurman *et al.*, 1991). Later in the season, when herbicide concentrations in rivers and streams are lower, water inflow into the reservoir is also reduced, limiting dilution of reservoir herbicide concentrations (Solomon *et al.*, 1996). Thus, while herbicide concentrations quickly drop in tributary streams following a storm event, concentrations may remain high in the receiving reservoir which, in turn, may release elevated concentrations of herbicides into stream(s) below the lake (Battaglin and Goolsby, 1998).

Atrazine is the second most widely used agricultural herbicide in the U.S., after glyphosate (Gianessi and Reigner, 2006), and is one of the most frequently detected herbicides in streams nationally (Gilliom *et al.*, 2006). In the Midwestern U.S., atrazine and other herbicides pulse through waterways in late spring/early summer following the pre-planting application to agricultural fields with the length and duration of pulses strongly affected by rainfall patterns. This phenomenon has been referred to as the "spring flush" (Thurman *et al.*, 1991, 1992), is not unique to North America (e.g., Europe: Palma *et al.*, 2009), and can occur for both high and low application-rate herbicides (Meyer *et al.*, 2007). Atrazine is particularly prevalent in the surface waters of the Midwestern U.S. In a survey of 122 hydrologic basins across 10 states within the corn-belt, atrazine was detected at 98% of sample sites during the post-planting season (May-June), with 55% of the basins exceeding the USEPA maximum contaminant level (MCL) for drinking water (3 μg/l) (Thurman *et al.*, 1992). More recently, Lerch *et al.* (2011) found that atrazine reached concentrations that may be harmful to aquatic organisms (USEPA, 2003) in a Missouri watershed during 10 of 15 years (1992-2006) studied. Five decades after atrazine was first registered for use, it continues to be a central component of weed management in the Midwest with few alternatives exhibiting equal economic and agronomic benefits for major crops such as field and sweet corn (Swanton *et al.*, 2007; Williams *et al.*, 2010).

The spring atrazine flush is likely to occur during an intense period of trophic interactions among fish, zooplankton, and phytoplankton. Dominant fish taxa (clupeids, centrarchids) spawn during late spring/early summer in the Midwest. The larval/early juvenile stages (young-of-year, YOY) of most of these fish species are zooplanktivorous (Dettmers and Stein, 1992). It is necessary for fish spawning and hatching to be synchronized with early-season peaks in zooplankton abundance to ensure an adequate food supply for fish larvae (Cushing, 1990), as growth rates of YOY fish are dependent upon availability of appropriate sized zooplankton in spring/early summer at the onset of exogenous feeding (Betsill and Van den Avyle, 1997; Bunnell *et al.*, 2003). Herbivorous zooplankton, in turn, are dependent upon phytoplankton as a food resource. As such, effects of atrazine on any one of these groups have the potential to cascade across many trophic levels in the aquatic food web.

Although atrazine use is targeted at control of broadleaf weeds, a large body of research has shown direct and indirect effects of atrazine on nontarget organisms. Complementing the primary research, the USEPA has developed aquatic life benchmarks for atrazine – concentrations below which the USEPA considers atrazine not likely to harm aquatic life (USEPA, 2011). Concentrations of 1-10 μg atrazine/l can reduce photosynthesis rates of phytoplankton and periphyton (Graymore *et al.*, 2001). Acute USEPA benchmarks are 1 μg/l for nonvascular plants (phytoplankton and periphyton) and 37 μg/l for vascular plants. Atrazine has also been shown to affect heterotrophs. Reported effects on biota include gonad deformities in amphibians at concentration thresholds of >0.1 μg/l (Hayes, 2004). Concentrations >0.5 μg/l interact with temperature resulting in altered sex ratios in turtles (Willingham, 2005). Concentrations above 1-5 μg/l have been reported to cause endocrine disruption in fish (Tillitt *et al.*, 2010). Concentrations >5 μg/l have been shown to cause an increase in male-to-female ratios of *Daphnia pulicaria* (Dodson *et al.*, 1999, 2000) and a variety of histological and behavioral changes in fish (Graymore *et al.*, 2001). Acute and chronic USEPA benchmarks for fish (2,650 and 65 μg atrazine/l, respectively) and invertebrates (360 and 60 μg atrazine/l, respectively, excluding reptiles and amphibians) are higher than atrazine concentrations reported to cause sublethal effects in the previously mentioned studies. Atrazine concentrations above 10-20 μg/l are likely to cause population and community-level effects in aquatic systems (USEPA, 2003, 2011), such as shifts toward dominance of resistant phytoplankton species (Graymore *et al.*, 2001). The chronic aquatic community benchmark for atrazine is 17.5 μg/l as defined by the USEPA (2011), but 1.8 μg/l as set by the Canadian Council of Ministers of the Environment (1999).

In addition to ecological concerns, atrazine in surface waters raises concerns with regards to drinking water supplies. While surface waters are subject to regulatory criteria under the Clean Water Act, water

that has been treated and piped to consumers is subject to regulatory criteria under the Safe Drinking Water Act. Surface water supplies that exceed the MCL for atrazine may need to be treated before being piped to consumers (or not used at all) to be in compliance with the Safe Drinking Water Act. The current MCL for drinking water is 3 μg/l under the Safe Drinking Water Act (USEPA, 2009). As of 2004 there were 53,437 community water supplies in the U.S., supplying water to the same population year round. Of these, 11,746 used surface water sources (e.g., reservoirs, streams, etc.) and supplied water to 62% of the U.S. population (Tesfamichael and Kaluarachchi, 2004). Traditional water quality monitoring performed under the Safe Drinking Water Act, using a low frequency of sampling performed over a short period of time, may underestimate chronic and intermediate risks to infants and children (Tesfamichael and Kaluarachchi, 2004). To better understand the tradeoffs between health risks and economic benefits of atrazine, there is a great need for studies that not only document atrazine concentrations in surface waters, but also determine factors affecting them at regional and local levels (Tesfamichael *et al.*, 2005).

Previous studies suggest that reservoirs and their receiving streams may exhibit lower peak herbicide concentrations but longer periods of elevated herbicide concentrations than unregulated streams (Battaglin and Goolsby, 1998). However, few studies have documented this phenomenon. Of these, most have investigated mechanisms affecting transport and degradation of atrazine within reservoirs (e.g., Fallon *et al.*, 2002; Chung and Gu, 2003, 2009; Ma *et al.*, 2008). Case studies quantifying the degree and consistency to which reservoirs alter exposure times relative to specific atrazine thresholds are rare, but important because the magnitude of ecological effects is likely to be strongly dependent upon both concentration and duration of the atrazine pulse as it moves through the reservoir. The timing and duration of this pulse determine the exposure concentrations relevant to specific life-history stages of fish (gravid adults, mature gametes, developing embryos, larvae, and early juveniles). Furthermore, because of the short generation times of zooplankton (days to weeks) and phytoplankton (hours to days), extended and elevated atrazine concentrations in the reservoir water column may affect planktonic organisms not only across multiple life-history stages, but across multiple generations. In this study, we focus on three thresholds (3, 5, and 10 μg/l) relevant to human and aquatic ecosystem health. We intensively sampled atrazine over the course of four years in a Midwestern stream/reservoir system surrounded by agricultural land, and address the following questions:

1. Does atrazine consistently flush into the reservoir during the spring of all four years?
2. Does the atrazine flush occur during a likely period of intense trophic interactions among phytoplankton, zooplankton, and YOY fish?
3. To what degree does the receiving reservoir extend exposure times above relevant thresholds compared to the major inflowing stream?
4. Does the persistence of elevated atrazine concentrations within the reservoir combined with regulated reservoir discharges result in longer exposures above threshold values in the outflow stream compared to the major inflow stream?

We did not seek evidence of ecotoxicological effects of atrazine in this study. Rather, we sought to characterize the ecological timing of the spring atrazine flush and the degree to which reservoirs may extend exposure times of aquatic organisms and drinking water sources above relevant atrazine concentration thresholds.

MATERIALS AND METHODS

Study Site

Acton Lake is a eutrophic reservoir located in southwestern Ohio (39°31′N latitude, 84°46′W longitude) and is designated as an emergency drinking water supply source for the city of Oxford, Ohio. It has a surface area of 232 ha, and a mean depth of 3.4 m. The watershed area draining into Acton Lake is 257 km^2, and dominated by agricultural (88.8%) land usage – primarily row crops such as corn and soybean (Knoll *et al.*, 2003). Acton Lake is long and narrow, following the contours of the flooded stream valley, with few side embayments (Figure 1). The reservoir was formed in 1958 via the impoundment of Four Mile Creek, a third order stream. Four Mile Creek drains approximately 50% of the Acton Lake watershed, with two other tributaries – Little Four Mile Creek, and Marshall's Branch – draining 30 and 5%, respectively (Vanni *et al.*, 2001). The remaining 15% of the watershed is dominated to a lesser extent by agriculture (53% in row crops) and contains Acton Lake itself and several small, ephemeral tributary streams. Acton Lake empties directly into Four Mile Creek below the dam. In this paper, upper Four Mile Creek refers to the inflowing portion of the stream, while lower Four Mile Creek refers to the portion downstream of the reservoir (Figure 1).

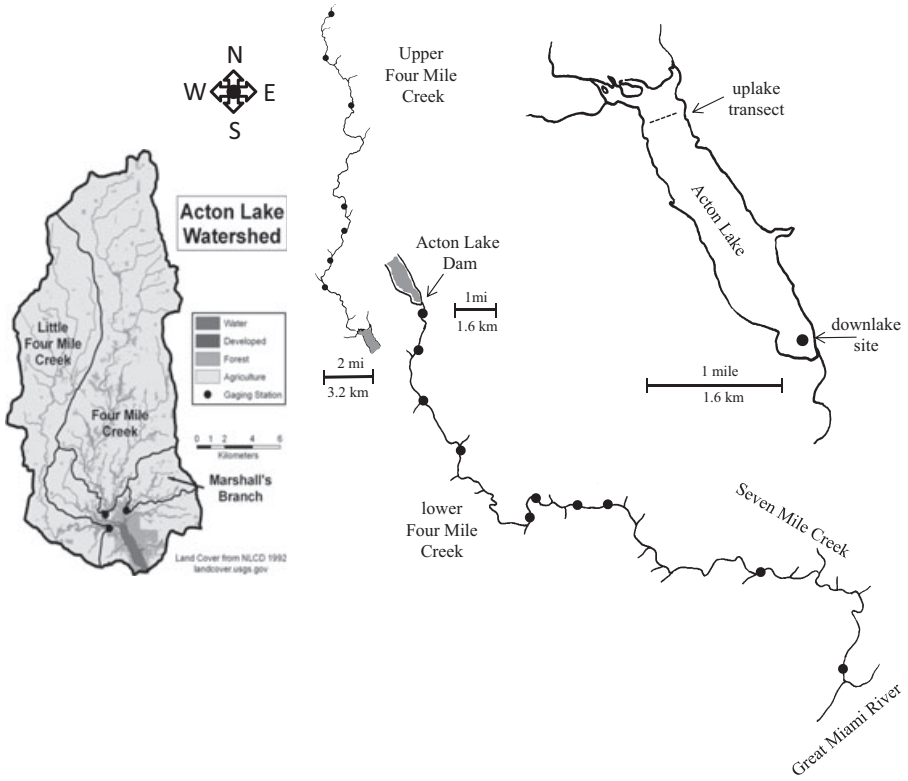

FIGURE 1. Map of Acton Lake Watershed, Upper Four Mile Creek and Associated Tributaries, Lower Four Mile Creek and Associated Tributaries, and Acton Lake. Circles represent locations of gaging stations on the watershed map and sampling locations on the Four Mile Creek maps.

Seasonal Dynamics of Reservoir Organisms

To assess general seasonal patterns of phytoplankton, zooplankton, and larval fish abundance in Acton Lake we examined records of organism abundance from 2003 to 2006 collected as part of an ongoing NSF Long Term Research in Environmental Biology monitoring program in the reservoir. All biotic samples reported in this study were collected from the downlake site of the reservoir. Phytoplankton abundance was quantified via chlorophyll *a* analysis of whole water samples from the euphotic zone (surface to the depth at which irradiance was equal to 1% of surface irradiance). For each sample, 25-50 ml of water was passed through a Gelman A/E glass fiber filter (Pall Gelman, Ann Arbor, MI) to retain algal cells. Chlorophyll *a* was extracted with acetone, and fluorescence of the extracted sample measured before and after acidification using a TD-700 fluorometer (Turner Biosystems, Sunnyvale, CA) calibrated with commercial standards dissolved in acetone (Knoll *et al.*, 2003).

Zooplankton was sampled by lifting a 63 μm plankton net through the entire water column. Samples were preserved with a 10%, buffered, sugar formalin solution (Prepas, 1978), returned to the lab, analyzed for abundance under 200× magnification (Stoeckel *et al.*, 2008) and identified to genus and species where possible. For the purposes of this study, we report abundances of three general zooplankton taxa: rotifers, copepod nauplii, and *Daphnia* spp.

Larval and early juvenile fish, hereafter referred to as YOY, were collected using a 500 μm mesh neuston net towed just under the surface at the downlake site at a rate of 1 m/s for 3 min per sampling date, preserved in 95% ethyl alcohol and returned to the lab for enumeration. All YOY fish were counted in samples containing fewer than 300 fish whereas samples containing >300 fish were subsampled using a plankton splitter. Fish were identified at least to the four main families found in the reservoir (Clupeidae, Centrarchidae, Cyprinidae, Percidae), and to species when possible (Auer, 1982). As gizzard shad comprised 80-90% of the total YOY fish catch within each year, we report only gizzard shad data in this paper and consider peaks in YOY gizzard shad abundance to represent temporal peaks in zooplanktivory. YOY gizzard shad are obligate zooplanktivores feeding sequentially on rotifers, nauplii, and cladocerans (e.g., *Daphnia*) as they grow (Bremigan and Stein,

1994; Miranda and Gu, 1998). Zooplanktivory by gizzard shad can be strong enough to depress reservoir zooplankton populations (Schaus *et al.*, 2002), creating a competitive bottleneck through which other fish species must recruit (Dettmers and Stein, 1992).

Analytical Technique for Atrazine

Atrazine is moderately soluble in water (33 ppm at 25°C) with an octanol-water partition coefficient (log P_{OW}) of 2.7645 at 20°C, a density of 0.35 g/ml, and vapor pressure of 40 μPa at 20°C (USEPA, 2003). We estimated atrazine concentration in each sample using USEPA-approved Atrazine RaPID Assay® immunoassay kits (Ohmicron Environmental Diagnostics/Strategic Diagnostics, Newark, DE). Minimum detection level was 0.046 ppb, with quantitation between 0.1 and 5 μg/l. Samples that returned values above 4.5 ppb were diluted with dilution media supplied with each kit until atrazine concentrations were reduced to levels within the quantitation range. Enzyme-linked immunosorbent assay (ELISA) techniques provide a valuable alternative to labor intensive and expensive gas chromatography/mass spectrometry (GC/MS) techniques (Lydy *et al.*, 1996; Adams *et al.*, 2004), allowing for more frequent and comprehensive sampling regimes when financial resources are limited.

The major limitation of ELISA is cross-reactivity: although the RaPID Assay is calibrated to atrazine reaction rate, it also reacts with eight other triazines (terbutryn, terbuthylazine, ametryn, prometryn, propazine, prometron, simazine, and cyanazine) and three atrazine breakdown products (desethyl atrazine, deisopropyl atrazine, and 6-hydroxy atrazine). Organic matter, chorination, quenching, and other factors can also decrease the accuracy of ELISA kits, including the RaPID Assay (Adams *et al.*, 2004). In general, previous studies have found a strong correlation between ELISA and GC/MS estimates of atrazine concentrations, with ELISA kits generally exhibiting a positive bias (Lydy *et al.*, 1996; Adams *et al.*, 2004; Byer *et al.*, 2011) although a negative bias can occur (Thurman *et al.*, 1992; Adams *et al.*, 2004). We are aware of three studies (i.e., Lydy *et al.*, 1996; Adams *et al.*, 2004; Byer *et al.*, 2011) in which measurements of atrazine in Midwestern surface waters were compared between RaPID Assay kits and GC/MS analysis. In two studies atrazine concentrations measured by RaPID Assay kits showed strong correlations to concentrations derived from GC/MS analysis of the same samples ($R^2 = 0.94$, $n = 149$, range = 0.02-15 μg/l, Lydy *et al.*, 1996; $R^2 = 0.88$, $n = 23$, range = 0.1-1.6 μg/l, Byer *et al.*, 2011). Correlations were not provided in Adams *et al.*(2004). In all studies, RaPID Assay kits tended to produce a positive bias – yielding atrazine concentrations higher than those determined from GC/MS analysis. Regression equations published in these papers indicate RaPID Assay estimates were 20% (Lydy *et al.*, 1996) and 23% (Byer *et al.*, 2011) higher than GC estimates of 1 μg atrazine/l, and 15, 13, and 12% higher than GC estimates of 3, 5, and 10 μg atrazine/l (Lydy *et al.*, 1996) when not corrected for cross-reactivity. Adams *et al.* (2004) found that RaPID Assay yielded atrazine concentrations that averaged 0.31 μg/l higher than GC/MS analysis. In addition, Adams *et al.* (2004) found that the 5 μg/l standards supplied in the RaPID Assay kits actually contained only 3.25 μg/l – a discrepancy that would account for overestimation of atrazine by RaPID Assay kits. The manufacturer (SDI) was alerted and the problem fixed immediately in 2003 (Adams *et al.*, 2004).

In this study, we compared ELISA to GC/MS estimates of atrazine in two sets of stock solutions. The first stock solution was made as part of a separate study (Stoeckel *et al.*, 2008) by dissolving an excess of atrazine (Chem Service, West Chester, PA; catalog PS 380, Chemical Abstract Service No. 1912-24-9) in 1 l of artificial freshwater (AFW: 10 ml of Instant Ocean [28-30‰], 990 ml of reverse osmosis water, 150 mg of CaCl, and 100 mg of $Na(CO_3)_2$; final pH 8.0 ± 2°C, salinity 0.5-1‰) and stirring for several hours in the dark. The resultant stock solution was filtered through 0.2 μm Whatman Anotop filters to remove undissolved atrazine. Four solutions for analysis were made by bringing 1, 3, 4.5, and 6 ml of stock solution up to 1 l AFW. Duplicate samples of each solution were analyzed by RaPID Assay and the remaining solution shipped to the Mississippi State Chemical Laboratory for GC/MS analysis. The second stock solution was made by dissolving 9.9 mg atrazine in 10 ml of ethanol and diluting up to 1 l with AFW. The resultant stock solution was filtered through 0.2 μm Whatman Anotop filters and four solutions for analysis were made in the same manner as for the first stock solution. Unfortunately, the 1 ml stock/999 ml AFW solution was dropped and broken, so we were only able to compare the remaining three solutions.

As the first stock solution was made with an excess of atrazine, we did not know the "true" concentration of atrazine. Results from the RaPID Assay (13.5 ± 1.5 μg atrazine/l) and GC/MS (10.8 μg atrazine/l) showed close agreement at the lowest test solution (1 ml stock 999 ml AFW), thus we used the average of the two techniques (12.16 μg atrazine/l) as the nominal value and calculated the nominal values of the remaining test solutions by multiplying 12.16 μg atrazine/l by 3, 4.5, and 6, respectively. For

the second stock solution, we set the nominal value of the lowest test solution (1 ml stock: 999 ml AFW) at 9.9 μg atrazine/l given that we had dissolved a known mass of atrazine in an ethanol carrier.

For solutions made with the first stock solution, mean RaPID Assay estimates of atrazine concentrations (two replicate samples from each test solution) fell on or just slightly higher than the 1:1 line between estimated and nominal atrazine concentrations, whereas GC/MS values consistently fell below the 1:1 line with increasing discrepancies at higher nominal values (Figure 2A). For solutions made with the second stock solution, RaPID Assay estimates from two separate analyses (run 1 and run 2) of atrazine concentrations fell consistently on or above the 1:1 line. Results from the GC/MS analysis fell consistently below the 1:1 line with distance from the line increasing with increasing nominal atrazine values (Figure 2B).

Samples from the uplake and downlake reservoir sites collected on June 8, June 22, and July 14, 2006 were also sent to the Mississippi State Chemical Laboratory for analysis. Results showed that atrazine concentrations estimated with the RaPID Assay kits were consistently higher than GC/MS estimates from the Mississippi lab, especially at higher atrazine concentrations. The degree to which RaPID Assay kits overestimated "true" atrazine concentrations in the reservoir is difficult to ascertain. Analysis of field samples via GC/MS is generally recommended to validate ELISA results (Byer *et al.*, 2011). However, the stock solution results (Figures 2A and 2B) suggest that the GC/MS analysis may have underestimated "true" atrazine concentrations. If so, this underestimation was not likely due to breakdown of atrazine in the stock solutions. Atrazine breakdown products (desethyl atrazine and desisopropyl atrazine) were not detected in any of the stock solution samples by GC/MS analysis.

Interference from cross-reacting breakdown products and/or other triazines were also not likely to have accounted for the full differences between RaPID Assay and GC/MS results in the reservoir samples. GC/MS analysis revealed desethyl atrazine and desisopropyl atrazine were present in all six reservoir samples tested. However, both of these compounds have a much lower percent-reactivity relative to atrazine (22 and 0.3%, respectively) (USEPA, 2007). The IC_{50} (concentration required to inhibit one half of the color produced by the negative control) used to calculate cross-reactivity values is 0.72 for atrazine, 3.21 for desethyl atrazine, and 217 for desisopropyl atrazine. On June 8, GC/MS analysis estimated desethyl atrazine concentrations as 2.0 ± 0.1 and 1.2 ± 0.1 μg/l in the uplake and downlake sites, respectively. Desisopropyl atrazine concentrations were 0.93 ± 0.05 and 0.75 ± 0.03 μg/l in the uplake and downlake sites, respectively. These products were not present in high enough concentrations to account for the large discrepancy in atrazine concentrations between RaPID Assay and GC/MS results on that sampling date (Figure 2C). Five cross-reacting triazines (ametryn, prometryn, cyanazine, propazine, and simazine) were below GC/MS detectable limits in all six reservoir samples. Samples were not analyzed for terbutryn and terbutylazine but these have a low

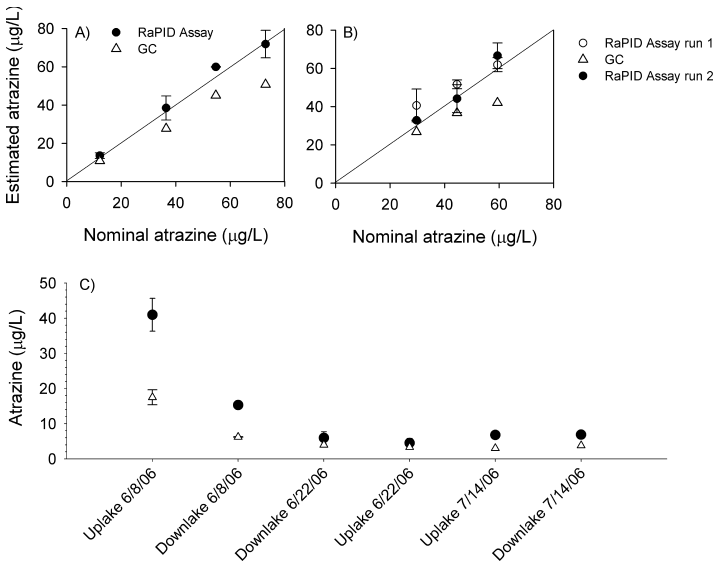

FIGURE 2. Atrazine Concentrations Measured by RaPID Assay and GC Analysis *vs.* Nominal Atrazine Concentrations for Standard Solutions Prepared with an (A) Excess of Atrazine and No Carrier, and (B) a Known Mass of Atrazine and an Ethanol Carrier. (C) Atrazine concentrations measured by RaPID Assay and GC analysis in reservoir samples collected in 2006. Error bars in all figures represent ±1 SD.

percent-reactivity relative to atrazine (13 and 5%, respectively) (USEPA, 2007).

Quality control parameters for the sample batch in which the June 8 samples were analyzed show an R^2 of 0.997 for the RaPID Assay calibration curve (a separate curve was constructed for each batch of samples run) and an estimate of 3.2 ± 0.39 μg/l for the nominal 3 μg/l atrazine standard supplied with the kit. Therefore, the discrepancy between RaPID Assay and GC/MS results were not likely due to a faulty RaPID Assay kit or to analytical errors.

Based on results of previous studies and our own comparative samples, atrazine concentrations measured by RaPID Assay kits and reported in this study are likely to be high estimates – true atrazine values were probably lower than reported values. However, the magnitude and consistency of the discrepancy between RaPID Assay estimates presented in this paper and "true" atrazine concentrations is unclear. The difference between estimates yielded by the two techniques (ELISA and GC/MS) for the June 8, 2006 reservoir samples (Figure 2C) was likely due to a combination of cross-reactivity, underestimation of atrazine by GC analysis, and possibly some other interference factor, making it difficult to determine the "true" atrazine concentrations in the reservoir.

Atrazine Sampling in the Main Tributary (Upper Four Mile Creek)

We collected stream samples for atrazine analysis in upper Four Mile Creek (the main inflow to Acton Lake) from 2003 through 2006. Stream samples were collected with an ISCO sampler <1 km upstream of the reservoir (see Vanni *et al.*, 2001) (Figure 1). The duration and frequency of sample collection varied between years due to financial constraints and technical problems with the sampler, but typically samples were collected every one to two days between April and September, or until atrazine concentrations had fallen below 3 μg/l for at least three weeks (Table 1). On each sampling day, we usually collected one sample per day during base flow and two to three samples per day (every 8-12 h) during pulses in stream discharge due to rain events. Differentiation between base flow and pulse flows was subjective and based on water height, flow, and clarity. Time of sample collection(s) during each day varied from week to week. As per RaPID Assay test kit directions, samples were filtered through 0.2 μm Anotop filters (Anatop™ 25 Plus; Whatman, Inc.: proprietary alumina based Anopore membranes) to remove suspended solids. Filtered samples were stored in amber glass bottles at 4°C, and analyzed within seven days of collection.

To estimate exposure days above 3, 5, and 10 μg atrazine/l we graphed atrazine concentration through time and counted the number of days above each threshold during a given sampling season. For days when atrazine data was missing, we assumed a linear relationship between the previous and next sampling event and estimated daily atrazine concentrations accordingly. This assumption may have led to some error in our estimated exposure times, but we feel that this error was minimized for the following reasons. For each of four years, the median interval between sampling days in upper Four Mile Creek throughout the sampling season was one day and the mean sampling interval was less than two days (Table 1). In 2003 and 2006, the maximum sampling interval prior to the final recorded atrazine concentration >3 μg/l was only three days. In 2004, the

TABLE 1. Summary of Sampling Seasons, Percent Days Sampled, and Sampling Intervals in Upper Four Mile Creek (U4M), the Uplake Reservoir Site, and the Downlake Reservoir Site from 2003 to 2006.

			Mean (median) Interval Between Sampling Days	Range in Sampling Intervals ≥2 Days	
Year	Site	Sampling Season (% days sampled)	Full Season	Full Season	Season Prior to Final >3 μg/l Estimate
2003	U4M	5/5-9/28 (64)	1.5 (1)	2-12	2-3
	Uplake	5/5-9/23 (54)	1.9 (1)	2-10	2
	Downlake	5/5-9/23 (48)	2.1 (1)	2-10	2-4
2004	U4M	4/10-6/27 (73)	1.4 (1)	2-9	2-9
	Uplake	4/15-7/20 (22)	5.0 (4)	2-17	2-8
	Downlake	5/12-7/3 (19)	7.4 (7)	6-9	6-9
2005	U4M	3/31-9/21 (71)	1.4 (1)	2-16	2-6
	Uplake	4/14-10/17 (20)	5.3 (4)	2-28	2-7
	Downlake	4/14-10/17 (18)	5.8 (4)	2-28	2-12
2006	U4M	4/19-8/2 (94)	1.1 (1)	1-3	3
	Uplake	4/14-9/28 (23)	4.6 (4)	1-21	2-8
	Downlake	4/28-9/28 (22)	4.6 (4)	1-21	2-8 (29)

maximum sampling interval was nine days. This interval occurred in May while flow was declining to baseline levels, thus we would expect atrazine levels to also be declining during this time. In 2005, the maximum interval prior to the final >3 μg/l measurement was six days and occurred in June during baseline flow when atrazine values were typically stable and not prone to spikes. A subsequent 16 days sampling interval occurred in July that bracketed a peak flow event (Figure 3). It is possible that we missed a spike in atrazine during this time, but atrazine concentrations did not spike during July in the other years of the study (Figure 3).

To confirm that Four Mile Creek was the main source of atrazine to Acton Lake, we measured atrazine concentration and discharge of the three major inflowing tributaries (Little Four Mile Creek, Four Mile Creek, and Marshall's Branch) from April 10 through June 29 in 2004. Atrazine collection and processing were conducted as described previously, with samples collected on 76, 71, and 68% of the potential sampling days for Little Four Mile Creek, Four Mile Creek, and Marshall's Branch, respectively. Mean daily discharge was estimated using techniques described in Vanni *et al.* (2001). Stage was recorded at each gaging station at 10 min intervals using pressure transducers installed in stilling wells, connected to dataloggers. Stage was converted to discharge with standard rating-curve techniques using field discharge measurements (Kennedy, 1983). Atrazine load was calculated by multiplying average daily atrazine concentration by daily discharge estimates on those days when both atrazine concentration and discharge estimates were available. Atrazine load was not calculated for those days when either concentration or discharge estimates were not available.

Atrazine Sampling in the Reservoir (Acton Lake)

Whole water samples were collected via an impeller pump in the upper reservoir, approximately 0.25 km downstream of the upper end of the reservoir near the tributaries (uplake site), and lower reservoir approximately 0.20 km upstream of the dam (downlake site) for four years (Figure 1). Sampling duration and frequency in the reservoir are summarized in Table 1. In 2003, we attempted to collect samples on a daily basis from May 6 to July 25, and once to twice weekly thereafter through September. Atrazine values fell below the lowest threshold of concern (3 μg/l) in the reservoir (June 25) while we were still sampling on a daily basis. During that time, we had one sampling interval ≥2 days at the uplake site and five sampling intervals ≥2 days at the downlake site (see Table 1 for additional information). Samples were collected once to twice weekly from April to July in 2004, and from April to September in 2005 and 2006. In 2004, mean interval between sampling days was 5.0 days at the uplake site and 7.4 days at the downlake site. Maximum sampling interval before reservoir atrazine had dropped below 3 μg/l was eight days at the uplake site and nine days at the downlake site. In 2005, the mean interval between sampling days was 5.0 and 5.5 days at the uplake and downlake sites, respectively. The maximum sampling interval before reservoir atrazine had dropped below 3 μg/l was 7 and 12 days for the uplake and down-

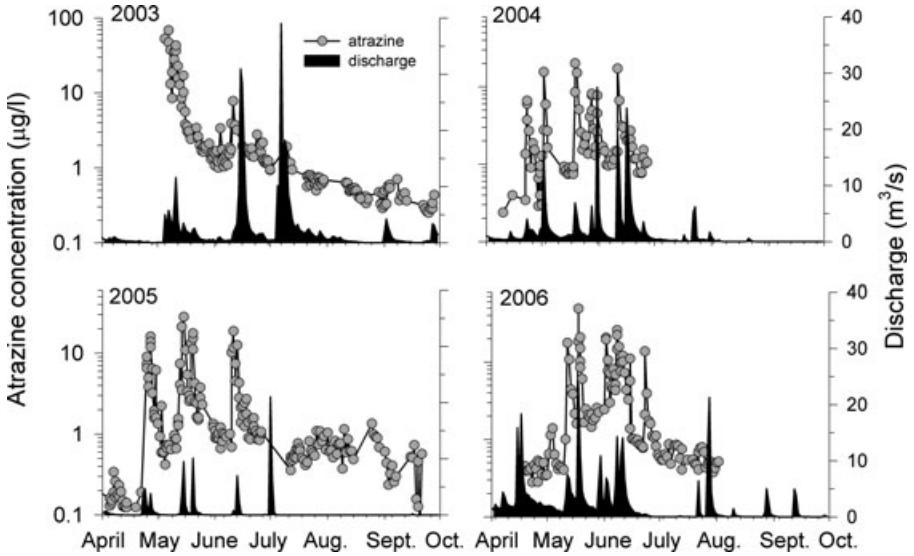

FIGURE 3. Atrazine Concentration and Discharge Values in Upper Four Mile Creek from 2003 to 2006. Measurements were taken at the gaging station site, just upstream of where Four Mile Creek empties into the reservoir.

lake sites, respectively. In 2006, the mean interval between sampling dates was 4.6 at both the uplake and downlake sites. Maximum sampling interval before reservoir atrazine had dropped below 3 μg/l was eight days at both the uplake and downlake sites. Exposure days were estimated in the same manner as for upper Four Mile Creek, and we assumed a linear relationship in atrazine concentrations between sampling events. Daily sampling in 2003 revealed that atrazine concentrations in the reservoir were much more stable than in upper Four Mile Creek, thus the typical (mean and median) sampling intervals of less than six days in 2004-2006 were not likely to result in large errors in exposure day estimations within the reservoir.

At each sampling site, duplicate 4 l, depth-integrated water samples were collected from the euphotic zone (from the surface to the depth at which irradiance was equal to 1% of surface irradiance). The euphotic zone generally comprised the entire water column (~1 m) at the uplake site and approximately 2 m at the downlake reservoir site. The euphotic zone never extended below the thermocline. We therefore assumed that the atrazine concentrations in the euphotic zone were representative of the entire, well-mixed, epilimnion.

To determine if water from tributaries had completely mixed in the upper reservoir, we collected duplicate composite samples from five evenly spaced sites across a ~400 m transect (Figure 1) on each sampling date in 2003 and 2004. Comparisons between the composite transect sample and mid-transect sample on two dates in 2003 and one date in 2004 revealed no significant differences in atrazine concentration (paired t-test: $p = 0.3$, $n = 3$) suggesting water was well mixed at this site. Therefore, due to personnel and budget constraints, we collected duplicate samples from a single, fixed-site midway along the transect in 2005 and 2006. At the downlake site (Figure 1), we collected samples from a single, fixed-site midway between the left and right descending shorelines (~0.2 km from the dam overflow) during all four years.

To test for elevated concentrations of atrazine in the hypolimnion during and after the spring herbicide flush, we collected water samples from the hypolimnion and euphotic zone at the downlake site in 2004 and 2006. Water temperature was recorded every 0.5 m from the surface to 5 m using a YSI model 58 DO/temperature meter (YSI, Inc., Yellow Springs, OH). In 2004, the majority of samples were collected during and immediately after the flush, whereas in 2006, most samples were collected after the flush. In both years, the metalimnion (zone of rapid temperature change with increasing depth) consistently ended at 5 or 6 m with temperatures below 6 m changing very little with depth. Therefore, we considered atrazine concentrations from 6 to 8 m to be representative of the hypolimnion. In 2004, we used a Van Dorn sampler to collect duplicate atrazine samples every 0.5 m in the euphotic zone and at 6, 7, and 8 m in the hypolimnion. Average atrazine concentrations were then calculated for the euphotic and hypolimnetic zones on each sampling date. In 2006, we used an impeller pump to collect duplicate integrated water samples from the euphotic zone and from the hypolimnion (6-7 or 8 m depending on reservoir depth) to determine average atrazine concentrations in each zone. All reservoir samples were returned to the lab in glass, amber bottles, processed, and analyzed for atrazine as described previously.

Atrazine Sampling in the Outflowing Stream (Lower Four Mile Creek)

In 2005, we collected samples in the outflowing stream of Acton Lake (lower Four Mile Creek) just below the reservoir spillway from May through September. We assumed the water was well mixed at this site and collected samples 0.25 m under the stream surface. Water samples were filtered and analyzed for atrazine as described previously.

To estimate the spatial extent of reservoir effects on downstream atrazine concentrations, we surveyed atrazine upstream, within, and downstream of the reservoir over a three-day period in June 2005. On June 28, we collected eleven samples along the entire stretch of lower Four Mile Creek (~34 km) from the reservoir to its confluence with the Great Miami River. On June 30, we collected seven samples along a 24 km reach of upper Four Mile Creek, two samples from the reservoir at the uplake and downlake sites, and a reference sample in lower Four Mile Creek for comparison to the sample collected two days earlier. Samples in upper and lower Four Mile Creek were collected from public access sites (bridges, parks, etc.) spaced as evenly as possible. Duplicate samples were collected 0.25 m under the surface at each site, filtered, and analyzed for atrazine as described previously.

Statistical Analyses

In this study, we estimated the number of exposure days above a specific atrazine concentration threshold by assuming a linear relationship in atrazine concentration between sampling events (see previous section). However, because the use of interpolated data may have led to some errors, we also calculated the odds of atrazine concentrations

exceeding a given threshold (i.e., 3, 5, and 10 μg/l) in the creek and reservoir in each of the four study years. For this analysis, we only used dates when samples were collected and did not utilize interpolated data. We tested whether the odds of atrazine concentrations exceeding a given threshold were different among the euphotic zone of upper Acton Lake, lower Acton Lake, and the major inflowing stream (i.e., upper Four Mile Creek) during the periods sampled from 2003 to 2006. To compare the odds of atrazine levels exceeding each of these thresholds, separate logistic regression models including the effect of site, year, and the interaction between site and year was used for each threshold level. Logistic regression transforms otherwise bounded (i.e., 0-1), nonlinear probabilities to continuous, linear log-odd ratios (i.e., $-\infty$ to ∞) via the logit link function (Agresti, 2002; SAS Institute, 2008). The logit model used for this analysis weights observations by the number of days a site was sampled, thus correcting for the unequal number of sampling events among the sites. Structure of the model was as follows:

$$\log_e[p/(1-p)] = \alpha + \beta_1 \times \text{site} + \beta_2 \times \text{year} + \beta_3 \times \text{site} \times \text{year}, \quad (1)$$

where p is the probability of atrazine levels exceeding a given threshold (i.e., frequency of sampling days above a given threshold/total sampling days for each site and year combination), α is the intercept or minimum log odds ratio of exceeding a given atrazine threshold excluding the effects of site, year, and their interaction, and β_1, β_2, and β_3 are coefficients determining the effect-size of site, year, and the interaction between site and year on the log odds ratio, respectively. If the Wald-chi-square statistic indicated that the main or interaction terms were significant, contrast statements were used to determine which levels were different. Results were then back-transformed to odds ratios and/or probabilities for interpretation purposes.

To test for differences in euphotic zone atrazine concentrations between the uplake and downlake sites during each of the four years, we utilized a paired t-test. At the downlake site in 2004 and 2006, we tested the strength of linear correlation between atrazine concentrations in the euphotic zone and hypolimnion via Pearson correlation, and tested for differences in atrazine between the two zones via paired t-tests. Data appeared to be normally distributed in 2004 (Anderson-Darling Test: $T = 0.263$, $p > 0.15$) and in 2006 (Anderson-Darling Test: $T = 0.414$, $p > 0.15$).

To test for differences in atrazine concentration between the major inflowing stream, Acton Lake, and the outflowing stream, we used paired t-tests ($\alpha = 0.05$) to compare atrazine concentrations at each site in 2005. All t-test and Pearson analyses were run using SYSTAT® Software, 2007 Ver 7.0 (SYSTAT Software, San Jose, CA) at a significance level of $\alpha = 0.05$.

RESULTS

Seasonal Dynamics of Reservoir Organisms

During all four years, gizzard shad YOY abundances peaked during the post-planting period (May-June) and subsequently fell to low/undetectable abundances in July. The rise in abundance reflects spawning and hatching of eggs, whereas the decline reflects a combination of mortality, and gear avoidance via increased swimming capacity. Zooplankton followed similar patterns with maximum abundances of all three major taxa generally peaking just before or during the post-planting season (May-June). Nauplii and *Daphnia* occasionally exhibited a second abundance peak in the fall (October). Chlorophyll *a* abundances were more variable, but showed a general pattern of increase during the post-planting season in all four years (Figure 4).

Exposure Days in Upper Four Mile Creek and Acton Lake

Atrazine concentration in upper Four Mile Creek increased with an increase in discharge during most storm events in the post-planting season (May, June) in all four years of the study (Figure 3). In 2004, Marshall's Branch exhibited the highest atrazine concentrations, with a maximum of 70 μg/l recorded in late May (Figure 5A). However, Four Mile Creek was the primary source of atrazine loading during the spring herbicide flush, delivering the highest daily loads of atrazine to the reservoir during peak loading events (Figure 5B).

Atrazine concentrations in upper Four Mile Creek and Acton Lake exceeded the 3 and 5 μg/l thresholds during the sampled periods in all four years of the study. Although atrazine concentrations quickly fell in Four Mile Creek after specific thresholds had been exceeded, concentrations remained above threshold values for a longer period in the reservoir (Figure 6; Table 2). In 2003, 2004, 2005, and 2006, the number of estimated exposure days above the 3 and 5 μg/l thresholds was consistently greater in the uplake and downlake sites than in upper Four Mile Creek

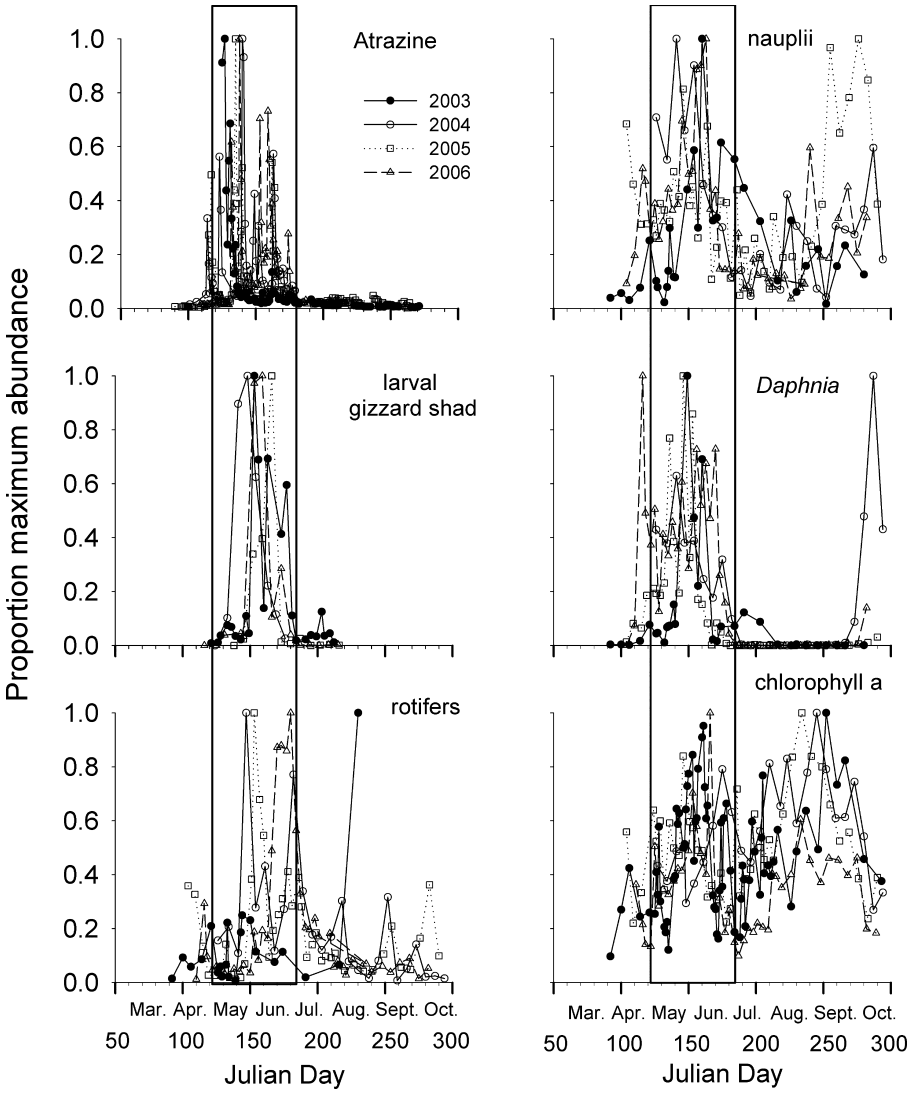

FIGURE 4. Proportion of Maximum Abundance (concentration) for Atrazine, Larval Gizzard Shad, Rotifers, Nauplius Larvae (copepods), *Daphnia*, and Chlorophyll *a* at the Downlake Reservoir Site During All Four Sampling Seasons of the Study. Box indicates the postplanting period (May, June) when the spring atrazine flush is most likely to occur.

(Table 2). It would have been optimal to sample both the creek and reservoir on a daily basis, but this was not practical across all four years. In 2003, we sampled upper Four Mile Creek and the reservoir as frequently as possible (Table 1: median sampling interval = 1.0 day for creek and 1.0 day for reservoir, mean sampling interval = 1.5 days for creek and 1.9 days for reservoir). Atrazine concentrations appeared to be relatively stable in the reservoir, but fluctuated strongly in the creek (Figure 6). Therefore, we continued to sample the creek on a daily basis whenever possible, but relaxed the sampling interval in the reservoir to once or twice per week (Table 1). As samples were collected more frequently from upper Four Mile Creek than from Acton Lake, it is possible that the number of exposure days would have been more similar between the creek and reservoir had we sampled both with the same frequency. However, when interpolation was restricted to dates on which both the creek and reservoir were sampled, exposure times remained higher in the reservoir than in the creek (Table 2).

Similar to estimates of exposure days, the odds of exceeding specific atrazine concentration thresholds were higher in the reservoir than in upper Four Mile Creek. This analysis was limited to data from sampling days and did not utilize interpolated data. Because it weighted observations by the number of days a site was sampled, it corrected for unequal numbers of sampling events between sites. At the 3 and 5 μg/l thresholds, logistic regression models indicated no significant interaction between site and year

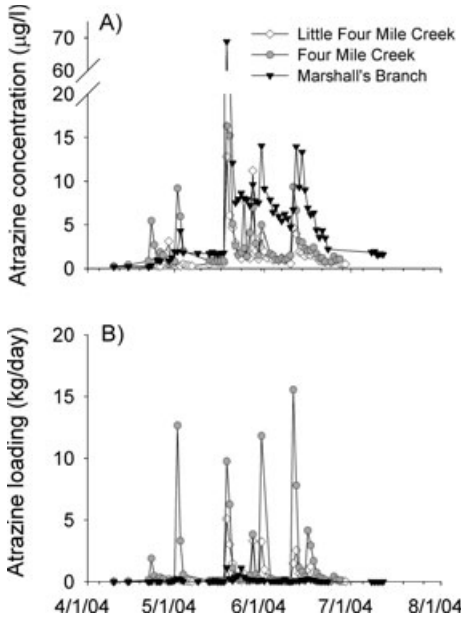

FIGURE 5. (A) Atrazine Concentrations in the Three Major Tributaries of Acton Lake from April Through July 2004. (B) Daily load of atrazine supplied to Acton Lake by the three main tributaries from April through July in 2004.

($p = 0.43$ and 0.40, respectively). Thus, the interaction term was dropped from both models. After dropping the interaction term, effect of year was not significant for the 3 µg/l threshold data and was also dropped from that model ($p = 0.52$). The final model, including only the effect of site, indicated differences among sites in the odds of exceeding 3 µg/l of atrazine ($p \leq 0.0001$). The uplake site was 5.2 times more likely to exceed the 3 µg/l threshold than the upper Four Mile Creek site ($p \leq 0.0001$) and the downlake site was 5.8 times more likely to exceed the 3 µg/l threshold than the upper Four Mile Creek site ($p \leq 0.0001$). The downlake and uplake sites had equal odds of exceeding the 3 µg/l threshold ($p = 0.64$).

After dropping the interaction term (site × year) from the 5 µg/l model, both site ($p < 0.0001$) and year ($p = 0.01$) significantly affected the odds of exceeding 5 µg/l. The uplake site was 4.5 times more likely to exceed the 5 µg/l level than the upper Four Mile Creek site ($p < 0.0001$), the downlake site was 4.8 times more likely to exceed the 5 µg/l level than the upper Four Mile Creek site ($p < 0.0001$), and the downlake/uplake sites had equal probabilities of exceeding the 5 µg/l threshold ($p = 0.83$).

Atrazine concentrations exceeded 10 µg/l in upper Four Mile Creek during the sampled periods in all four years of the study, but in only two years (2003 and 2006) in the reservoir sites (Figure 6; Table 2). The logistic regression model indicated no significant interaction between site and year ($p = 0.97$) and no significant effect of site ($p = 0.13$) after the removal of the insignificant interaction term. The lack of a significant site effect is likely due to the fact that in 2004 and 2005, concentrations in the reservoir never exceeded 10 µg/l. However, within 2003 and 2006, when atrazine concentrations exceeded 10 µg/l in the reservoir the number of exposure days exceeding

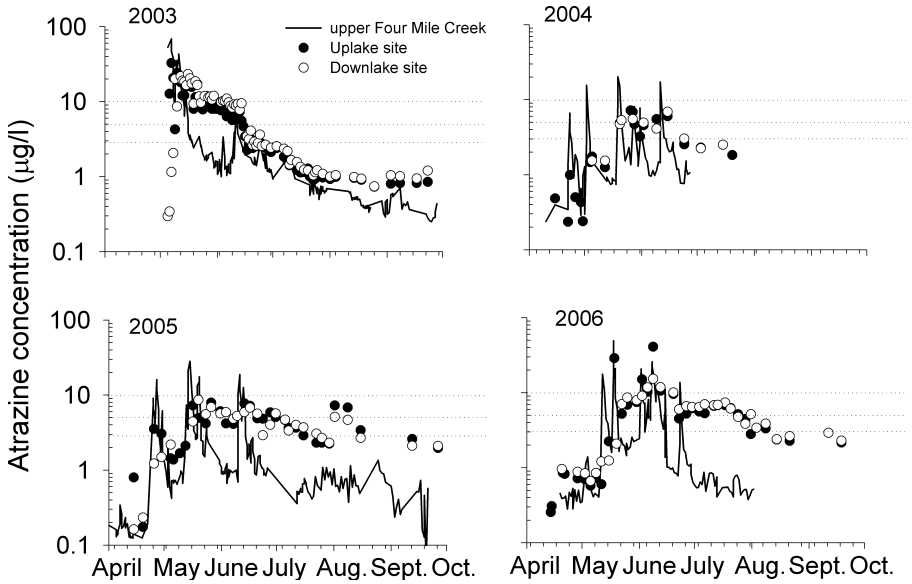

FIGURE 6. Atrazine Concentrations in Upper Four Mile Creek and Two Reservoir Sites from 2003 to 2006. The uplake reservoir site was located near the inflowing tributaries whereas the downlake reservoir site was located near the dam. Dotted lines indicate three atrazine threshold values discussed in this paper (3, 5, and 10 µg/l). Data points represent actual sampling data, not interpolated estimates.

TABLE 2. Exposure Days Above Three Thresholds of Atrazine Concentrations in Upper Four Mile Creek and Acton Lake.

Concentration Threshold (μg/l)	Sampling Year	Four Mile Creek (no. of days)	Acton Lake	
			Uplake site (no. of days)	Downlake site (no. of days)
≥3	2003	17 (15)	41	43
	2004	13 (22)	36	38
	2005	15 (17)	94	85
	2006	22 (23)	81	83
≥5	2003	12 (10)	39	38
	2004	7 (13)	20	18
	2005	12 (11)	39	35
	2006	16 (19)	65	62
≥10	2003	9 (8)	14	24
	2004	3 (4)	0	0
	2005	5 (5)	0	0
	2006	7 (10)	17	15

Notes: Exposure days for upper Four Mile Creek are calculated from either data interpolated between "all measurement dates" (no parentheses) or data interpolated only between "dates when Acton Lake was also sampled" (parentheses).

10 μg/l was greater in the reservoir than in upper Four Mile Creek (Table 2).

Atrazine appeared to be fairly well mixed both horizontally and vertically within the reservoir. We found no significant differences in atrazine concentrations between the uplake and downlake reservoir sites in 2003 ($p = 0.58$), 2004 ($p = 0.63$), 2005 ($p = 0.31$), or 2006 ($p = 0.57$) (Figure 6). We found no evidence for elevated concentrations of atrazine in the hypolimnion during and immediately after the spring flush in 2004 (Figure 7). Mean atrazine concentrations in the euphotic zone and hypolimnion were positively correlated ($r = 0.82$, $p = 0.002$) with no significant difference between mean atrazine concentrations in the two zones ($p = 0.262$). Weak evidence for elevated concentrations of atrazine in the hypolimnion was found in 2006 (Figure 8). Atrazine concentrations in the euphotic zone and hypolimnion

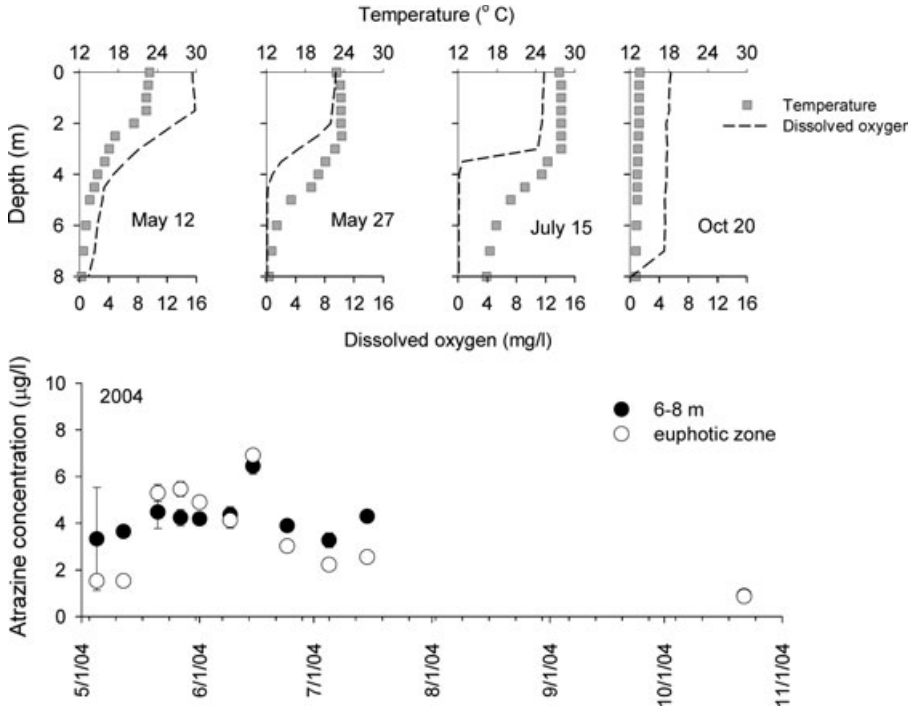

FIGURE 7. Depth Profiles of Temperature and Dissolved Oxygen and Mean Atrazine Concentrations in the Euphotic Zone and Hypolimnion (6-8 m) at the Downlake Site in 2004. Error bars represent ±1 SD.

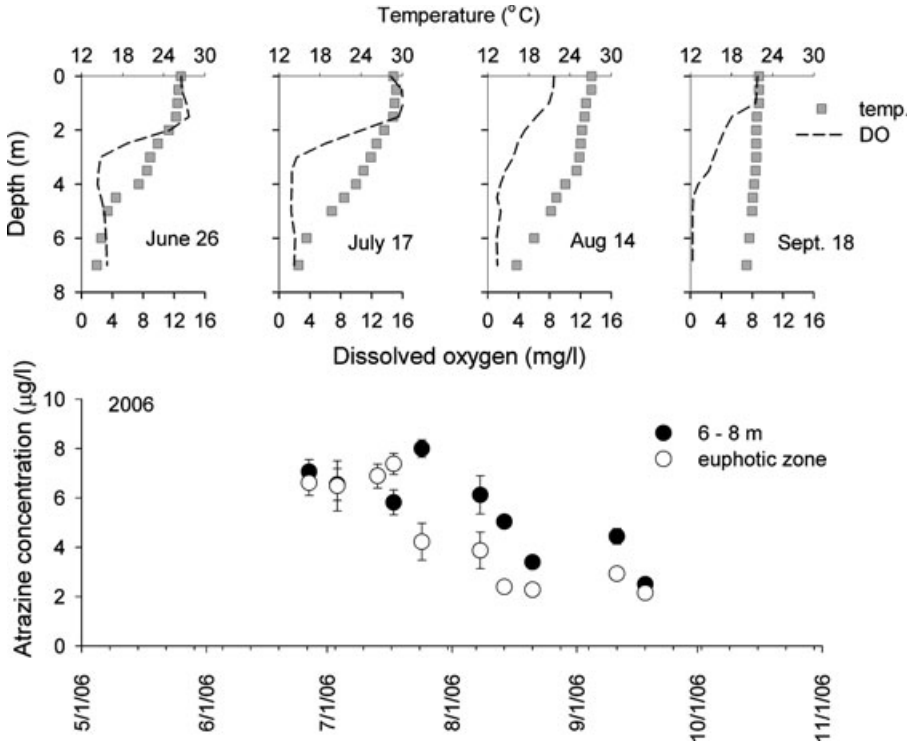

FIGURE 8. Depth Profiles of Temperature and Dissolved Oxygen and Mean Atrazine Concentrations in the Euphotic Zone and Hypolimnion (6-8 m) at the Downlake Site in 2006. Error bars represent ±1 SD.

were positively correlated ($r = 0.667$, $p = 0.051$), but atrazine concentrations between the euphotic zone and the hypolimnion at the downlake site were marginally different ($p = 0.06$); on 7 of the 10 sampled dates, mean atrazine concentrations were greater in the hypolimnion than in the euphotic zone.

Outflowing Stream

In paired samples collected from June through August in 2005, atrazine concentrations in lower Four Mile Creek were not significantly different from those observed at the downlake site ($p = 0.26$) but were significantly different from concentrations in upper Four Mile Creek (above the reservoir) ($p < 0.0001$). Furthermore, atrazine concentrations in lower Four Mile Creek remained elevated above those in upper Four Mile Creek from late June through at least mid-September when the 2005 sampling in upper Four Mile Creek was discontinued (Figure 9).

Longitudinal sampling during June 2005 along a 24 km reach of lower Four Mile Creek below the reservoir dam indicated a decrease in atrazine concentrations downstream (Figure 10). Atrazine concentration in lower Four Mile Creek quickly dropped from 6.6 µg/l ± 0.85 SD to 1.8 µg/l ± 0.03 SD over a ~7 km distance downstream of the dam, then leveled off at ~2 µg/l for the next 18 km, and dropped again to 0.7 µg/l ± 0.02 SD near the confluence of Four Mile Creek and the Great Miami River (Figure 10). Dilution by groundwater was a potential factor but was not measured during this study. Dilution by minor tributaries was not likely a major factor in the initial

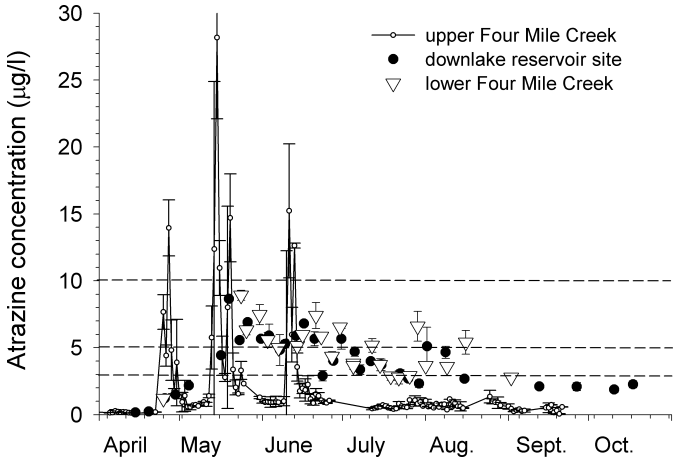

FIGURE 9. Mean Atrazine Concentrations in the Outflowing Stream (lower Four Mile Creek) as Compared to the Inflowing Stream (upper Four Mile Creek) and Reservoir. Error bars represent ±1 standard deviation. Dotted lines indicate the three atrazine thresholds considered in this study.

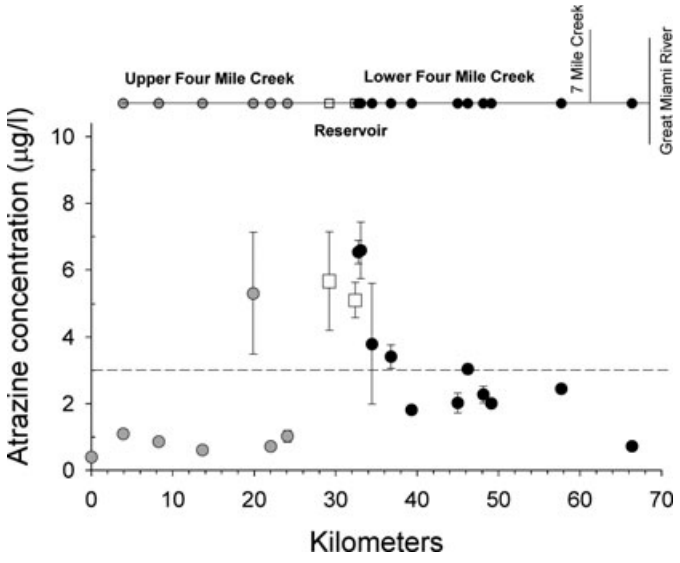

FIGURE 10. Mean Atrazine Concentrations Along the Major Inflowing Stream (upper Four Mile Creek), Reservoir, and Outflowing Stream (lower Four Mile Creek) in 2005. Error bars represent ±1 SD. Sites on lower Four Mile Creek were sampled on June 28, whereas sites along upper Four Mile Creek and in the reservoir were measured on June 30. The first site on lower Four Mile Creek (offset solid circles) was sampled twice for comparison between sampling days. Dashed line on graph represents the USEPA drinking water standard (3 µg/l) (USEPA, 2009).

decline. Three minor tributaries joined lower Four Mile Creek ≤7 km downstream of the dam as atrazine concentrations declined rapidly but atrazine concentrations did not show a further decline below ~2 µg/l even though an additional 11 minor tributaries joined Four Mile Creek over the next ~20 km (Figures 1 and 10). Either those minor tributaries also had atrazine concentrations near 2 µg/l or they did not have enough flow to significantly dilute atrazine in Four Mile Creek. However, after a major tributary (Seven Mile Creek) joined lower Four Mile Creek ~30 km downstream of the dam, atrazine concentrations declined below the 2 µg/l plateau (Figures 1 and 10), suggesting dilution effects by a major tributary just before Four Mile Creek emptied into the Great Miami River. For most of the ~30 km stretch in lower Four Mile Creek, atrazine levels were intermediate between the reservoir and upper Four Mile Creek.

Two days later we again measured atrazine in lower Four Mile Creek just below the reservoir dam, as well as sites along upper Four Mile Creek extending ~30 km upstream of the reservoir. Atrazine measured at the reference site in lower Four Mile Creek showed a nearly identical atrazine concentration compared with that measured two days earlier (6.5 µg/l ± 0.35 SD *vs.* 6.6 µg/l ± 0.85 SD). Samples collected from upper Four Mile Creek showed consistent concentrations less than approximately 1 µg/l, with the exception of one site 9 km upstream of the reservoir that yielded a mean concentration of 5.3 µg/l ± 1.82 SD (Figure 10). This site was sampled hours after the onset of a heavy rainfall event (see subsequent discharge event in Figure 3), and the samples (n = 2) were taken from a sediment plume coming from a minor tributary with a temporarily high discharge due to the rain.

DISCUSSION

Timing of the Spring Herbicide Pulse

Reservoirs are the dominant North American lake type at latitudes below 42° north (Thornton, 1990) and receive substantial inputs of nutrients, detritus, and herbicides draining from terrestrial landscapes. Ecologists now recognize the importance of investigating interactions between fluxes of terrestrial detritus/nutrient subsides and aquatic food webs (Thornton, 1990; Carpenter *et al.*, 1998; Polis *et al.*, 2004). However, much less attention has been paid by ecologists to the annual flux of herbicides and pesticides from terrestrial to aquatic systems.

In our study system the spring atrazine flush was an annual event, flushing down the main tributary during each of the four study years. Similar to many other reservoirs in eastern North America, Acton Lake is dominated by gizzard shad (*Dorosoma cepedium*), a species that can regulate reservoir ecosystems via impacts on other fish species, zooplankton, phytoplankton, and nutrient transport (e.g., Vanni *et al.*, 2005 and references therein). The spring atrazine flush occurred during a period of intense YOY fish/zooplankton/phytoplankton interactions as gizzard shad reproduction coincided with seasonal peaks in the prey items on which zooplanktivorous larval stages depend (Cushing, 1990; Betsill and Van den Avyle, 1997; Bunnell *et al.*, 2003) (Figure 4 this study). The spring flush also coincided with the spring increase in phytoplankton (Figure 4) upon which zooplankton grazers depend.

Atrazine thus had the potential to affect multiple life-history stages and cascade across multiple other levels. Because atrazine flushed into the reservoir during the spawning period of gizzard shad (Figure 4) and other fish taxa such as centrarchids (M.J. González, J.M. Duncan, and T. Lyon, unpublished data) a wide range of life-history stages (gravid adults, mature gametes, developing embyros, larvae, and early juveniles) were exposed. Similarly, because many zooplankton taxa produce continuous broods with short development times, multiple sequential

broods were likely subjected to the spring flush. For example, Stoeckel *et al.* (2008) estimated that approximately 13 sequential *Daphnia* broods (mean development time = 2.8 days) were exposed to atrazine levels >5 μg/l in 2003. The strength of the atrazine effects (if any) in this and other Midwestern reservoirs are likely strongly dependent upon the degree to which the reservoir dilutes the incoming atrazine, the degree that exposure times are increased due to storage of atrazine in the reservoir, and the rate at which atrazine breaks down in the reservoir.

Retention of Atrazine by the Receiving Reservoir

Many cities and towns use reservoirs as their primary or emergency (e.g., Oxford, Ohio and Acton Lake) drinking supply source, and the ubiquity of atrazine in Midwestern waterways has raised many human health concerns (Tesfamichael *et al.*, 2005; Tesfamichael and Kaluarachchi, 2006). Strong concerns have also arisen regarding effects of atrazine and other herbicides on aquatic ecosystems (e.g., Graymore *et al.*, 2001). Once herbicides such as atrazine are flushed into a reservoir, concentrations may remain at elevated for longer periods of time than under natural (i.e., unregulated) flow conditions (Battaglin and Goolsby, 1998).

Frequent sampling within the reservoir over a four year period allowed us to quantify the degree to which Acton Lake extended the exposure times above specific thresholds relative to human and ecosystem health. Although the reservoir dampened atrazine concentrations below the maximum concentrations observed in the inflowing stream (upper Four Mile Creek), atrazine concentrations in the reservoir did not decrease rapidly following the spring flush. Rather, they remained elevated above relevant thresholds for a much longer time in the reservoir than in upper Four Mile Creek. These extended exposure times were measured in the euphotic zone – the zone where atrazine is most susceptible to photodegredation (Chung and Gu, 2003). As Acton Lake was almost always thermally stratified during the summer months (Nowlin *et al.*, 2005) (Figures 7 and 8 this study) we hypothesized that exposure times may have been extended in waters below the thermocline due to retention of atrazine in the darker waters of the hypolimnion. However, our study did not indicate substantially higher atrazine concentrations in the hypolimnion compared to the euphotic zone – although there was some indication of marginally significant higher concentrations in the hypolimnion during sampling in 2006. Furthermore, the hypolimnion in Acton Lake was generally anoxic (Figures 7 and 8) and was unlikely to serve as a habitat for fish or other aerobic organisms. These results are similar to those of Fallon *et al.* (2002) who found that development of a metalimnion in a large Kansas reservoir had little effect on vertical atrazine distribution. Thus, in our study, extended exposure times in the euphotic zone were likely representative of concentrations and impacts on the entire reservoir community.

It is difficult to judge whether reservoir-mediated changes in exposure time had a negative effect on the reservoir's aquatic community. Even when exposure concentrations are well documented, results of ecotoxicological studies can often be contradictory and difficult to apply to natural populations. For example, Dodson *et al.* (1999, 2000) hypothesized that induction of excess male production in *Daphnia* by atrazine may reduce population growth rates at a time when *Daphnia* are subjected to heavy YOY fish predation. In contrast, we found that male production by *Daphnia parvula* in Acton Lake during the spring atrazine flush was not likely caused by atrazine (Stoeckel *et al.*, 2008). Olmstead and LeBlanc (2003) also found no evidence for increased male production by *Daphnia magna* exposed to atrazine. Thus, it does not appear likely that the spring herbicide flushes of atrazine result in depressed *Daphnia* populations due to excess male production.

However, atrazine has been shown to have a variety of effects on a wide range of organisms, with exposure time being an important determinant of the severity of those effects. Some periphyton (Gustavson *et al.*, 2003) and phytoplankton taxa developed resistance to herbicides (including atrazine) following chronic (e.g., 25 days) exposure (Seguin *et al.*, 2002) with resistant taxa becoming dominant while herbicides remain in the system. The phytoplankton community in Acton Lake may be subjected to high interannual variation in selection pressure, with a high selection for atrazine-resistant species occurring in some years but little pressure in others. Tillitt *et al.* (2010) showed that effects of atrazine on fish reproduction can be time-dependent. Significant effects of 0.5-5 μg atrazine/l on cumulative egg production were not apparent until 17 days of chronic exposure. In our study, exposure to atrazine concentrations ≥5 μg atrazine/l was prolonged over 20-67 days in the reservoir, providing sufficient time for the type of chronic effects documented by Tillitt *et al.* (2010) on fathead minnows. It is unknown whether atrazine has similar effects on ecologically dominant reservoir species such as gizzard shad. A literature search (Science Citation Index) using the search terms "atrazine" AND "gizzard shad" OR "dorosoma" OR "clupeidae" yielded no studies examining effects of atrazine on gizzard shad. Given the ubiquity of gizzard shad and reservoirs in the Midwestern U.S., and the important role gizzard shad play in regulating

reservoir ecosystems (Vanni *et al.*, 2005), future studies examining effects of atrazine on gizzard shad are warranted.

As an additional complication, the spring herbicide flush may contain multiple herbicides with the potential for interactive effects, particularly during chronic exposure. Following the spring flush into a Portuguese reservoir, Perez *et al.* (2010) found that *D. magna* did not suffer acute toxicity when exposed to reservoir water containing multiple pesticides (atrazine ≤5.5 μg/l), but suffered a significant decrease in number of offspring when subjected to chronic exposure. In the same system Palma *et al.* (2010) hypothesized that low concentrations of multiple herbicides observed in the reservoir (atrazine ≤2.5 μg/l, combined pesticides <25 μg/l) caused an observed depression in algal growth due to interactive effects. Communities in upper Four Mile Creek were consistently exposed to higher atrazine (and possibly other herbicide) concentrations, but for a much shorter time, suggesting that acute effects of herbicide runoff may be more important in upper Four Mile Creek whereas chronic effects may assume greater importance in the reservoir.

Effect of the Reservoir on Downstream Exposure Regimes

Pesticide exposure regimes of drinking water intakes and aquatic communities in streams may be strongly affected by their position relative to reservoirs, with reservoirs dampening maximum concentrations, but continuing to release atrazine-laden water to receiving streams long after the spring flush (Battaglin and Goolsby, 1998; Fallon *et al.*, 2002). In this study, exposure regimes of stream communities were mediated by location relative to the reservoir. Upstream communities were subjected to short-term exposure to atrazine levels above the relevant thresholds whereas downstream communities were subjected to chronic exposure. This pattern of short-term upstream exposure seemed to hold true for communities along most of a ~24 km reach upstream of the reservoir. Samples in this reach, collected after the last recorded atrazine pulse of the spring flush (see Figure 9), showed all but one sampling site contained <1 μg atrazine/l (Figure 10). The single elevated atrazine concentration (5.3 μg/l) observed in Four Mile Creek 20 km upstream of the reservoir was likely due to local elevated atrazine concentrations in storm runoff from a minor tributary, after the onset of heavy rainfall just prior to sample collection at this site. In contrast, the pattern of chronic downstream exposure to atrazine levels above the 3 and 5 μg/l did not persist longitudinally along a ~34 km downstream reach of Four Mile Creek as concentrations rapidly declined downstream from the reservoir. The mechanism for the initial rapid decline (~6 to ~2 μg/l) in atrazine concentrations is unclear but may have been influenced by dilution from groundwater or tributary stream inflows. Regardless of the mechanism behind the decline, aquatic communities were exposed to atrazine levels equal to those in the reservoir for only a short distance downstream of the reservoir.

SUMMARY AND CONCLUSIONS

The results of this study show that the spring herbicide pulse is now a predictable, anthropogenic seasonal event with relative location of reservoirs strongly affecting the potential of atrazine to affect aquatic communities. Our results show that reservoirs can prolong the time period over which organisms are exposed to concentrations above critical thresholds both within, and for a short distance downstream of reservoirs. Effects of increased duration of exposure to elevated herbicide concentrations due to reservoir storage are likely to be manifested in lentic communities within the reservoir and lotic communities downstream, but not upstream, of the reservoir. Although we focused on atrazine, additional triazines and herbicide degradation products are almost certainly present in many reservoirs. This study indicates that atrazine, and potentially other persistent herbicides, may be an important seasonal stressor with the potential to alter aquatic ecosystems, as well as to influence the timing and suitability of using reservoirs and their receiving streams as a safe drinking water resource in agricultural areas.

ACKNOWLEDGMENTS

This study was conducted while the lead author was a PhD student in the Department of Zoology at Miami University, Oxford, Ohio. The authors are grateful for the efforts of numerous undergraduate and graduate students who helped collect data for this paper, particularly R. Cooper, J. Elder, S. Noble, L. Schnurr, and A. Bowling. This research was funded by National Science Foundation DEB-0235755, NSF Research Experience for Undergraduate Grants to Miami University, Miami University Faculty Research Program Grants to MG and Miami University Department of Zoology Summer Field Workshop grants to JS. Funding sources had no involvement in the study design, collection and analysis of data, writing of the report, or the decision to submit the paper for publication. This manuscript was greatly improved through the efforts of three anonymous reviewers and their efforts are sincerely appreciated.

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