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[Article]

Effects of High Levels of Antimycin A on Aquatic Invertebrates in a Warmwater Arizona Stream

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Abstract.—Restoration of native fish to freshwater habitats often requires nonnative fish removal via chemicals such as antimycin A. Despite widespread use, there are limited field studies quantifying the effects of antimycin A on aquatic macroinvertebrates. We studied the immediate and short-term effects of antimycin A on macroinvertebrates during a fish renovation project in Fossil Creek, Arizona. We employed before–after control–impact (BACI) designs to measure the effects of antimycin A (at extraordinarily high levels of >54 and >100 μg/L) on macroinvertebrate drift, density, and species composition. We used the Hilsenhoff biotic index, a measure of invertebrate pollution tolerance, to study changes in species composition. At the highest dose (>100 μg/L), drift was five times the pretreatment drift level and invertebrate standing stocks in pools and riffles decreased immediately. Densities rebounded in riffles within 5 months but remained depressed in pools. At the lower concentration (>54 μg/L), macroinvertebrate mortality, measured as increased drift, was 24 times the pretreatment level. At this lower concentration, however, macroinvertebrate densities in the benthos were not reduced. Under both concentrations, species composition shifted toward more tolerant species. Although antimycin A effects were mostly short term, several species were locally extirpated. We found no explanation for the loss of some species over others. These results indicate that there is a high end concentration at which antimycin A can have deleterious effects on aquatic invertebrates. We caution managers contemplating the use of antimycin A in fish restoration to consider the risks to macroinvertebrates. We suggest the use of pretreatment surveys and bioassays at anticipated treatment levels to predict the effects upon macroinvertebrates, especially sensitive species. Where there are sensitive species, steps should be taken to mitigate effects.

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Nonnative species are implicated as one of the primary threats to freshwater biodiversity worldwide (Allan and Flecker 1993; Richter et al. 1997). Nonnative fish have displaced native fish throughout the southwestern USA, where the majority of native fish are listed as extinct, endangered, or threatened, or are candidates for listing (Cross 1978; Marsh and Minckley 1990; Anderson et al. 1995; Dudley and Matter 2000). The threat of nonnative species is often magnified by habitat degradation, leading managers to consider removing nonnatives in conjunction with habitat improvements (Brasher 2003; Ormerod 2003). Eradication of nonnative fish usually requires chemical treatment, although there have been some limited successes from intensive netting in lakes (Knapp and Matthews 1998) and intensive electrofishing in streams (Kulp and Moore 2000). Two commonly used piscicides for killing fish are rotenone and antimycin A, which both inhibit cellular metabolism of exposed organisms. Rotenone has been used since the 1930s, but severe impacts on nontarget organisms and the negative public perception of rotenone have led many fisheries managers to favor antimycin A, even though much less is known about its effects on other organisms.

Antimycin A, a fungal antibiotic, was discovered in the late 1940s, and its potential for use as a piscicide was recognized in the early 1960s (Walker et al. 1964). Like rotenone, it affects cellular metabolism and inhibits the electron transport chain in mitochondria, effectively stopping cellular energy production (Rieske et al. 1967). Antimycin A is preferred over rotenone as a fish toxicant because it (1) is toxic to fish at lower concentrations than rotenone, (2) degrades into nontoxic constituents within hours or days, (3) has low toxicity to nontarget organisms but targets fish effectively, (4) is undetectable to target organisms, and (5) can be used in a wider range of water temperatures than rotenone (Derse and Strong 1963).

Most of the evidence supporting antimycin A as a better piscicide is based on laboratory or small-scale field studies (Walker et al. 1964; Snow 1974; Houf and Campbell 1977) and a few field studies from large fish eradication projects (Gilderhus et al. 1969). Effects of antimycin A on nontarget organisms are still not fully

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understood. Although effects on other vertebrates (e.g., amphibians, reptiles, and mammals) seem to be minimal (Walker et al. 1964; Gilderhus et al. 1969; Greselin and Herr 1974), reports on aquatic invertebrates are varied (e.g., Morrison 1979; Minckley and Mihalick 1981). The prevailing notion, however, is that there are minimal effects and that any existing effects are short term. These perceptions are based largely on government publications that have not been peer reviewed and are often limited in availability (e.g., Walker et al. 1964; Gilderhus et al. 1969). Much of the research has been conducted using low concentrations of antimycin A (<10 μg/L), but real-world applications of antimycin A can exceed these concentrations by several fold. This study documents the effects of antimycin A, applied at extraordinary concentrations exceeding 50 μg/L, on the invertebrate assemblage in Fossil Creek, Arizona. It should be noted that these are extreme concentrations that may represent the highest known treatment application to date.

To study the effects of antimycin A, we employed a modified before–after control–impact (BACI) design comparing drift rates and benthic samples in two treated and one control site before and after chemical treatment. Invertebrate drift was used as an immediate measure of application effects; benthic samples from pools and riffles were used to measure immediate effects and longer-term impacts at 4 and 5 months posttreatment. We predicted that antimycin A would create high mortality, evidenced by higher drift rates during treatment and reduced standing stock immediately after treatment; we suspected that invertebrate densities would rebound within 6 months of treatment. We also hypothesized that the species composition of the community would shift to more tolerant invertebrates.

Study Site

Fossil Creek (Figure 1) is a perennial, travertine-depositing, spring-fed stream originating from a layer of Mississippian Naco limestone along the Mogollon Rim in northern Arizona. A series of seven springs (UTM Zone 12: 3809309 N, 447275 E; elevation = 1,304 m above sea level) creates the majority of the 1.302-m^3/s base flow, although scattered smaller springs along the length of the stream also contribute. This springwater contains large concentrations of calcium carbonate and dissolved carbon dioxide (Table 1). During our study, the majority of flow was diverted at a small diversion dam less than 1 km below the springs, but some seepage flow (<0.056 m^3/s) created a perennial stream except during severe droughts.

Nonnative fish were removed as part of a larger restoration program involving the decommissioning of the hydropower operation and restoration of flows in June 2005. The nonnative fish removal took place during the fall of 2004 before decommissioning so that it could be conducted under reduced flows. We conducted our study from August 2002 to March 2005 while the hydropower plant was still in operation.

The areas above and directly below the diversion dam were not treated because they lacked nonnative fishes. The predominant native fishes above the dam were the desert sucker *Catostomus clarkii*, speckled dace *Rhinichthys osculus*, and headwater chub *Gila nigra*. Below the dam, the native headwater chub was replaced by the native roundtail chub *G. robusta*, and the native Sonora sucker *C. insignis* was also encountered. Nonnative green sunfish *Lepomis cyanellus* extended from the confluence of the Verde River to approximately 1.6 km from the springs. The uppermost limit of this species marked the beginning of the treatment reaches. Nonnative smallmouth bass *Micropterus dolomieu* were abundant from the Verde River to the Irving Power Plant, where roughly 0.142 m^3/s of water was returned to the river, increasing flow to 0.198 m^3/s. Closer to the confluence with the Verde River, two more nonnative fishes were present: flathead catfish *Pylodictis olivaris* and yellow bullhead *Ameiurus natalis*.

Before restoration, Fossil Creek supported a diverse macroinvertebrate assemblage of over 135 taxa (Marks et al. 2005). Two species of special concern are found within the Fossil Creek drainage: the Page Spring microcaddisfly *Metrichia nigritta* (Trichoptera: Hydroptilidae), which occurs throughout Fossil Creek, and the Fossil springsnail *Pyrgulopsis simplex* (Gastropoda: Hydrobiidae), which is limited to Fossil Springs and several smaller springs within the drainage.

Methods

Renovation schedule and procedures.—Specific details about the treatment methodology are described by Weedman et al. (2005). However, a brief description of the renovation procedures is relevant. The Arizona Game and Fish Department (AZGFD) partitioned the stream into two separate reaches for renovation corresponding to the two flow regimes in the regulated portion of the stream. Treatment reach 1 started at the furthest known distribution of nonnative fish (~1.6 km below the spring) and ended downstream at a large waterfall at the Irving Power Plant. Treatment reach 2 started at the end of reach 1 and ended approximately 9.8 km downstream at a fish barrier constructed by the U.S. Bureau of Reclamation. Native fish were salvaged from both reaches before chemical treatment, transported by helicopter to a

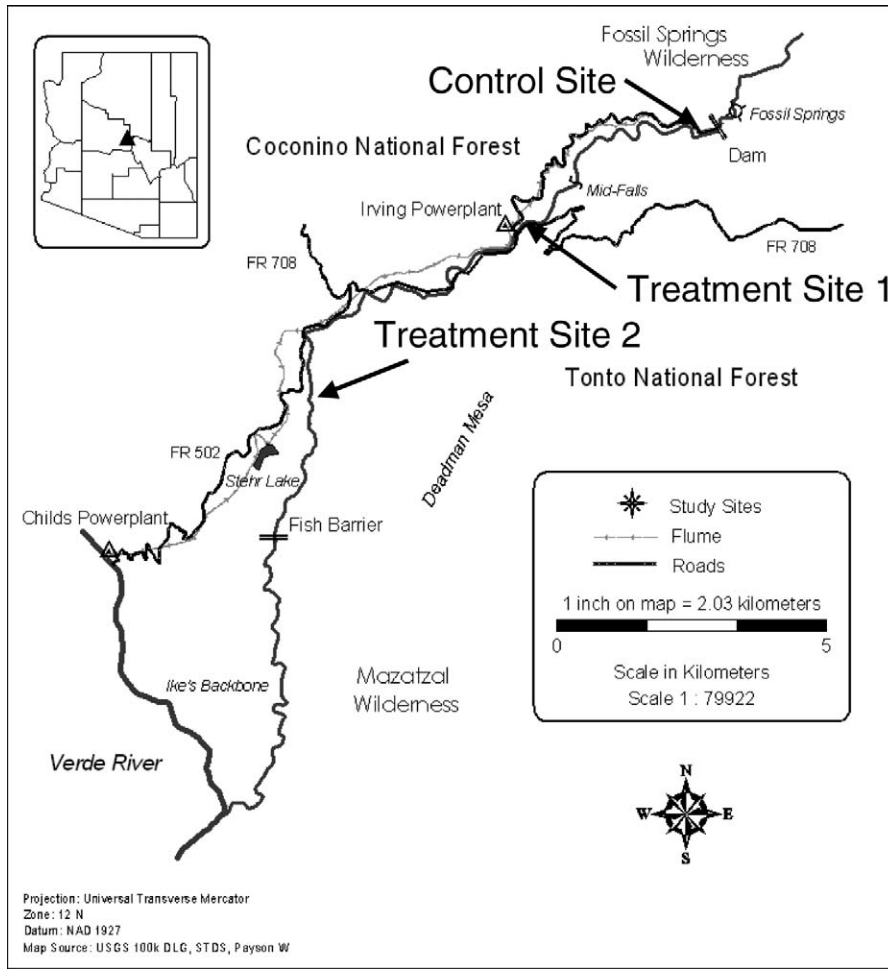

FIGURE 1.—Map of Fossil Creek, Arizona, showing study sites where antimycin A effects on aquatic macroinvertebrates were examined before and after treatments to remove nonnative fishes.

holding facility, and returned to the river after chemical treatment.

The first reach was treated with antimycin A (Fintrol) on 19–20 October 2004. Antimycin A target dosage was 50 μg/L in the approximately 0.014-m^3/s base flows in the main channel, but the bottom of the reach (lower 900 m) had a target dosage of 100 μg/L (increased due to high iron in the water). Additional treatment of side channels occurred at 10 μg/L. Application was accomplished using bucket drip stations, approximately 150 m apart, during a 4-h exposure period; drip application conformed to procedures recommended by the manufacturer. In addition to the drip stations, antimycin A was applied in two other ways. First, antimycin-A-laden sand (Fintrol 15) was added to deep pools to ensure full treatment of pools with slow turnover. Second, backpack sprayers added additional antimycin A to isolated water bodies, backwaters, and vegetated stream margins; renovation crews were instructed to approximate an application of 50 μg/L. Because of these additional application methods, final treatment concentrations of antimycin A experienced by the stream biota were probably in excess of the targeted application of 50 or 100 μg/L. Detoxification of antimycin A was accomplished using a drip station of potassium permanganate at 3 mg/L at the downstream end of the treatment reach.

Treatment of reach 2 was conducted in a similar manner with some exceptions. The upper portion of the reach was treated at an application concentration of 35 μg/L on 9 November 2004, when stream discharge was 0.481 m^3/s. It was then treated at a concentration of 54 μg/L on 11 November 2004, when stream discharge was 0.311 m^3/s. The lower section was treated on 10

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TABLE 1.—Physiochemical characteristics of antimycin-A-treated and control sites in Fossil Creek, Arizona. Values are means of samples taken seasonally from August 2002 to May 2003.

Variable	Control site	Treatment site 1	Treatment site 2
Temperature (°C)	19.44	17.92	17.67
Dissolved O_2 (mg/L)	7.13	7.59	7.65
pH	8.12	8.2	8.37
Conductance (μS/cm)	611.91	573.77	520.86
NH_3 (mg/L)	0.04	0.03	0.03
PO_4 (mg/L)	0.04	0.07	0.03
Salinity (‰)	0.34	0.29	0.26
NO_3-N (mg/L)	1.32	0.06	0.12
Mg (mg/L)	37.47	40.46	17.64
Ca (mg/L)	78.38	50.9	40.16
Na (mg/L)	10.97	14.37	11.63
K (mg/L)	1.73	2.24	1.79
Cl (mg/L)	7.34	9.09	7.71
SO_4 (mg/L)	22.41	15.34	24.06
CO_2 (mg/L)	32.67	27.35	28.34
Alkalinity	383	296	246
Secchi transparency (nephelometric turbidity units)	1.16	2.6	1.71

November 2004, when discharge was 0.311 m^3/s, resulting in an application of 54 μg/L. The lower section was treated at the planned concentration of 50 μg/L on 12 November 2004, when stream discharge was 0.311 m^3/s. Backpack sprayers and antimycin-A-laden sand were applied as described above. Detoxification at the end of the lower reach involved use of sodium permanganate applied at 3 mg/L instead of potassium permanganate.

The antimycin A concentrations used represent extraordinary treatment levels, well above the commonly used range of 5–15 μg/L. However, the physical and chemical variables and treatment timing necessitated the use of these levels. Bioassays on fish in Fossil Creek indicated the need for high levels of antimycin A, perhaps attributable to the high pH, temperature, and iron levels in the creek (Weedman et al. 2005). Furthermore, the scheduled restoration of full flows to Fossil Creek (1.218 m^3/s) in June 2005 prohibited a second chance for eliminating nonnative fish.

These levels of antimycin A are not only outside of the range of typical treatments but are substantially higher than label recommendations (up to 20 μg/L), which may exceed legal limits. However, the label allows for treatment outside this limit if bioassays indicate the need for higher levels and if the state game and fish agency grants permission. For Fossil Creek, both conditions were met (the treatment was performed by AZGFD), ensuring legality.

Site selection.—Site selection is an important aspect of environmental impact detection. We selected two impact sites and one control site that corresponded with long-term survey sites. As part of our monitoring program, all sites were sampled six times in the 2 years before restoration. In treatment reach 1, the impact site, which was directly above the Irving Power Plant, experienced target antimycin A applications of 100 μg/L. In treatment reach 2, the impact site was just downstream of the middle of the reach and received target antimycin A concentrations of 54 μg/L. These different treatment regimes allowed us to assess the impacts of antimycin A at two different levels.

One control site was used as a reference site for both treatment sites. The control site was 100 m below the diversion dam and experienced the same flow regime as treatment site 1. This site was sufficiently close to the impact sites that it experienced the same natural variation in flow and climate as the impact sites. We were constrained to one control site because downstream sites may have been contaminated and sites above the diversion dam had considerably different flow regimes.

Each site (control and treatments 1 and 2) was approximately 200 m in length, and all benthic and drift sampling was done in random locations within each reach.

Benthic sampling.—We sampled the benthic macroinvertebrate assemblages in both riffles and pools before and after the treatments (Table 2). Riffles were sampled using a 929-cm^2 Surber sampler with 250-μm mesh and following standard protocols (Hauer and Resh 1996). Samples were taken at impartial locations within the study reach. Invertebrates and substrate (cobble, gravel, and particulate matter) collected in the Surber sampler were transferred to a 20-L bucket and elutriated into another bucket to remove the inorganic matter. Invertebrates were preserved in 95% ethanol. Pool invertebrates were sampled using a 324-cm^2 core sampler, driven into the pool substrate as deep as possible at an impartially selected location. A trowel was used to retain the sample while transferring it to a 20-L bucket. Once in the bucket, samples were elutriated, preserved in 95% ethanol, and processed in the same manner as the Surber samples. The number of replicates of core and Surber samples depended on the sampling period. Five replicates were taken as part of long-term studies, and 10 replicates were taken in the periods leading up to and after the antimycin A treatment (Table 2). In the laboratory, invertebrates were sorted with a magnifying glass, identified to the lowest practical taxonomic level (usually genus), and enumerated.

Drift sampling.—Collection of drifting invertebrates followed standard protocols (Smock 1996) using two different net designs: one large design by a commercial manufacturer (Aquatic Ecosystems) and one small,

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TABLE 2.—Treatment and sampling schedule for antimycin A application in Fossil Creek, Arizona. Numbers in parentheses indicate the number of replicate samples on that date for benthos, the number applies equally to both core and Surber samples (e.g., 5 = 5 core and 5 Surber).

Site	Treatment concentration (μg/L)	Treatment date	Benthos		Drift	
			Before treatment	After treatment	Before treatment	During treatment
Control	0	None	14 Aug 2002 (5) 4 Dec 2002 (5) 1 Oct 2003 (5) 5 May 2003 (5) 31 Jan 2004 (5) 13 Oct 2004 (10)	19 Nov 2004 (10) 14 Dec 2004 (10) 17 Mar 2005 (10)	13 Oct 2004 (10) 8 Nov 2004 (10)	19 Oct 2004 (21) 10 Nov 2004 (16)
Treatment site 1	>100	19 Oct 2004	15 Aug 2002 (5) 16 Dec 2002 (5) 5 May 2003 (5) 30 Sep 2003 (5) 30 Jan 2004 (5) 18 Oct 2004 (10)	4 Nov 2004 (10) 13 Dec 2004 (10) 16 Mar 2005 (10)	18 Oct 2004 (10)	19 Oct 2004 (18)
Treatment site 2	>54	10 Nov 2004	12 Aug 2002 (5) 16 Dec 2002 (5) 5 May 2003 (5) 30 Sep 2003 (5) 30 Jan 2004 (5) 5 Nov 2004 (10)	13 Dec 2004 (10) 16 Mar 2005 (10)	5 Nov 2004 (10)	10 Nov 2004 (24)

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homemade design. The manufactured design measured 30 × 30 cm and was covered with 250- or 500-μm mesh netting. The homemade design was 14 × 14 cm and had 500-μm mesh netting. Nets were placed in riffles and secured in place using rebar driven into the stream substrate. The nets were left in the water column for approximately 120 min, after which we recorded current velocity and depth of the net. After nets were removed from the water, samples were washed into buckets and processed in a similar manner to benthic samples. Samples were washed through a sieve (1-mm mesh) to standardize for differences in net mesh size. Invertebrates were sorted, counted, and reported as the number individuals per 100 m^3 of water (Smock 1996). All drift samples were taken at similar times throughout the day, and we avoided dawn and dusk periods when behavioral drift might confound the results.

Because simple changes in density do not address changes in community structure, we used the Hilsenhoff biotic index (HBI) as an additional variable in our analyses. This index is a weighted average of tolerance values for each sample (Hilsenhoff 1987, 1988) and is calculated as

$$\text{HBI} = \sum n_i t_i / N,$$

where n_i is the number of individuals counted for species i, t_i is the tolerance value for species i, and N is the total number of individuals in a sample. Tolerance values range from 0 (stress intolerance) to 10 (high stress tolerance). Tolerance values for invertebrates in our samples were taken from regional values developed for the U.S. Environmental Protection Agency (Barbour et al. 1999). Because no values have been developed for the southwestern USA, we used the values developed for the Pacific Northwest or the Midwest. In the rare case when no value existed for a taxon, we omitted that taxon from the HBI. Because these values were developed to measure the tolerance of organisms to organic pollutants, we feel that they are relevant and indicative of antimycin A effects. Increases in HBI values after treatment indicate a shift in community composition to more tolerant species.

Statistical analyses.—Short-term HBI and density changes for both benthos and drift were analyzed with analysis of variance (ANOVA) using a standard BACI model (Green 1979). This method uses both time (before–after) and site (control–impact) as factors in the model, but significance of the impact is revealed in the interaction of site and time. For the analysis of the drift, we modified this test, replacing "after" with "during" (i.e., before–during control–impact). All density data used in the analyses were transformed ($\log[x + 1]$) to normalize variances.

We used a BACI paired series (BACIPS) test to measure the long-term impacts (Stewart-Oaten 1996). It uses paired differences in the control–impact sites as the dependent variable and time (before–after) as the sole factor. Because our drift samples were collected before and during the treatment, this analysis was not applied to the drift. All ANOVAs were performed using JMP IN (version 4.02).

Where there were significant or marginally significant effects in either the short or long-term HBI, we visualized the assemblages using the nonmetric

TABLE 3.—Results of ANOVA tests examining the effect of antimycin A treatment on changes in invertebrate density and the HIlsenhoff biotic index (HBI) measured at treatment site 1, Fossil Creek, Arizona. The ANOVA types are before–after control–impact (BACI) and BACI paired series (BACIPS). For BACI tests, only the site × date interaction is given. Effects were measured during treatment (immediate) and at 2 weeks (short term) and 5 months (long term) posttreatment.

Sample	Treatment effect	ANOVA type	Response variable	df	*F*	*P*
Drift	Immediate	BACI	Density	1, 55	4.076	0.048
			HBI	1, 53	2.372	0.129
Riffle benthos	Short term	BACI	Density	1, 39	8.413	0.007
			HBI	1, 39	18.062	0.001
	Long term	BACIPS	Density	1, 8	0.985	0.354
			HBI	1, 8	0.341	0.577
Pool benthos	Short term	BACI	Density	1, 39	4.642	0.038
			HBI	1, 39	0.305	0.585
	Long term	BACIPS	Density	1, 8	5.583	0.051
			HBI	1, 8	0.920	0.370

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multidimensional scaling (NMS) ordination routine in PC-ORD (version 4.02). These ordinations provide a graphical representation of community differences using the Sorenson (Bray–Curtis) distance measure. In the case of drift, we relativized the data to the sample maximum and thereby adjusted for potentially large density differences between samples collected before and during treatment. To assist in the interpretation of these ordinations, we used the joint plot function of PC-ORD and the following secondary variables: total species richness (SR), evenness, Shannon's diversity index *H*, Simpson's diversity index *D*, coleopteran SR, dipteran SR, ephemeropteran SR, trichopteran SR, other (miscellaneous taxa) SR, and HBI. When the joint plot is run, it creates a directional vector that shows the relationship between the secondary variables and the ordination scores. If a vector points toward a certain group of samples, those samples are positively correlated with the secondary variable; PC-ORD also calculates a coefficient of determination r^2 associated with the secondary variable.

A common concern is that antimycin A may result in the local extirpation of species that will fail to recover. To this end, we performed indicator species analysis in PC-ORD to detect whether there were any species that were only present in the benthos before treatment and that had failed to either persist or recover after treatment. In interpreting the results, we considered only species that were present immediately before treatment but were absent in 100% of the posttreatment samples. Significance of the indicator species analysis was tested using a Monte Carlo randomization test in PC-ORD. Because absence of certain taxa could be explained by seasonal differences (e.g., emergence), we also performed the indicator species analysis on the control site as a comparison.

Results

Treatment Site 1

There were significant short-term treatment effects of antimycin A in treatment site 1 (Table 3). Density of drifting invertebrates significantly increased from 83.5 individuals/100 m^3 before treatment to 443.2 individuals/100 m^3 during treatment, a more than fivefold increase (BACI ANOVA: $F_{1,55} = 4.075$, $P = 0.048$; Figure 2A). Observations of drifting invertebrates indicated that the majority were dead upon collection. The HBI values estimated during treatment were not significantly different from pretreatment values ($F_{1,53} = 2.372$, $P = 0.129$).

Invertebrates were scarce in benthic samples taken soon after treatment. Density of benthic invertebrates in riffles significantly dropped from 2,802 individuals/m^2 before treatment to 300 individuals/m^2 after treatment (BACI ANOVA: $F_{1,39} = 8.41$, $P = 0.007$; Figure 2B). Riffle HBI also increased from 4.98 to 6.98 ($F_{1,39} = 18.062$, $P < 0.001$), indicating a shift to more tolerant species. The effects in riffles were mirrored in pools, where density exhibited a fivefold decline from 3,162 individuals/m^2 before treatment to 610 individuals/m^2 after treatment ($F_{1,39} = 4.624$, $P = 0.038$). There was no effect of treatment on HBI in pools ($F_{1,39} = 0.035$, $P = 0.585$; Figure 2C).

Macroinvertebrates mostly rebounded within 5 months posttreatment. There were no detectable long-term effects either in density or HBI in riffles (Table 3). Five months after treatment, riffle invertebrates increased to 3,326 individuals/m^2, a value slightly higher than the immediate pretreatment value (2,802 individuals/m^2). The HBI similarly rebounded to values approximating pretreatment levels within 2 months. Pool invertebrates also increased from 610 individuals/m^2 (posttreatment low) to 1,410 individuals/m^2 by 5 months posttreatment. Although densities had not yet

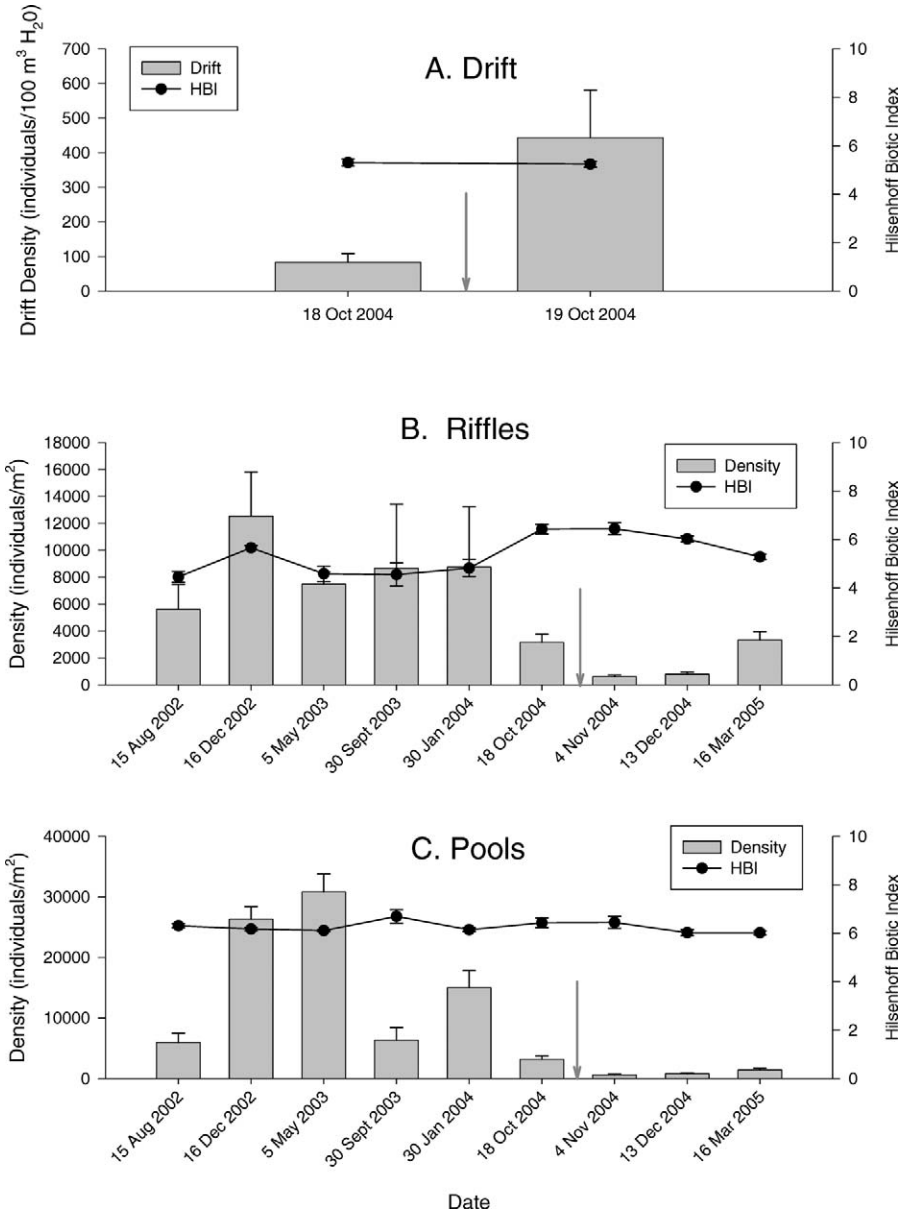

FIGURE 2.—Mean (SE) macroinvertebrate **(A)** drift density, **(B)** benthic density in riffles, and **(C)** benthic density in pools within Fossil Creek, Arizona, treatment site 1 before and after antimycin A application (arrow indicates date of treatment) to remove nonnative fish. The Hilsenhoff biotic index (HBI) is presented in each panel. Drift density was measured during instead of after treatment.

recovered to pretreatment levels, the test for long-term effects was only marginally significant (BACIPS ANOVA: $F_{1,8} = 5.583$, $P = 0.051$).

Because there was a detectable shift in the HBI in the invertebrate riffle assemblage, we ran NMS ordination to visualize the shift in community (Figure 3). The ordinations show a clear grouping of samples taken shortly after treatment that was not apparent before treatment or at 5 months posttreatment, indicating that the assemblages changed after treatment and then recovered close to a pretreatment state. Furthermore, the joint plot explanatory variables with an r^2 higher than 0.4 included HBI tolerance values (0.59), ephemeropteran diversity (0.42), and SR (0.48). In

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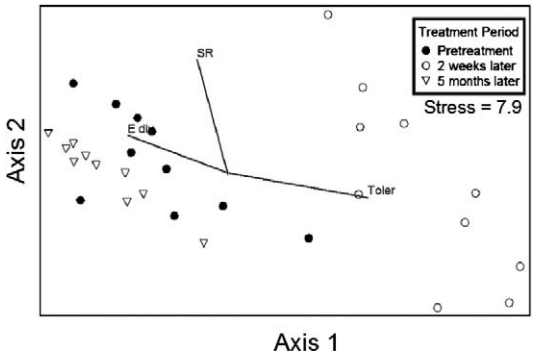

FIGURE 3.—Nonmetric multidimensional scaling ordination of invertebrate assemblages in riffles at treatment site 1, Fossil Creek, Arizona, showing relationship among samples taken before antimycin A application (used for nonnative fish removal) and at 2 weeks and 5 months posttreatment. Joint plots are variables that explained more than 40% of the variability ($r^2 > 0.40$) along axis 1 or 2: Hilsenhoff biotic index tolerance values (toler), species richness (SR), and ephemeropteran diversity (E div).

this configuration, higher tolerances are associated with the samples taken shortly after treatment, whereas samples taken pretreatment and at 5-months posttreatment are associated with increased SR and ephemeropteran diversity.

Treatment Site 2

Like treatment site 1, application of antimycin A at site 2 resulted in immediate large increases in density of drifting invertebrates (BACI ANOVA: $F_{1,57} = 31.582$, $P < 0.001$; Table 4; Figure 4A). The increase at site 2 was much larger than that at site 1 and was almost 24 times (556.3 individuals/100 m^3) the pretreatment value (23.2 individuals/100 m^3). Visual inspection again indicated that the majority of invertebrates were dead upon collection. Although the HBI of drift was not significantly different ($F_{1,53} = 3.85$, $P = 0.055$), HBI tended to be lower for invertebrates drifting during treatment (average = 4.76) than before treatment, suggesting that less-tolerant species were more strongly affected by chemical treatment (average = 5.48).

Unlike treatment site 1, there was no short-term effect of antimycin A application on density either in riffles (BACI ANOVA: $F_{1,39} = 0.023$, $P = 0.881$) or pools ($F_{1,39} = 0.037$, $P = 0.848$; Table 4; Figure 4B, C). Not surprisingly, there was no long-term effect either in riffles (BACIPS ANOVA: $F_{1,7} = 0.007$, $P = 0.937$) or pools ($F_{1,7} = 2.841$, $P = 0.144$). There were, however, short-term and long-term effects on the riffle invertebrate assemblage as measured by HBI (BACI ANOVA: $F_{1,39} = 11.884$, $P = 0.002$; BACIPS ANOVA: $F_{1,7} = 10.717$, $P = 0.017$), indicating that the community shifted to more tolerant taxa.

Ordination of drift assemblages separated pretreatment drift from treatment drift (Figure 5). The joint plot function shows that the pretreatment drift had higher HBI tolerance values ($r^2 = 0.37$) than those drifting during treatment, indicating that less-tolerant organisms (as measured by HBI) are more susceptible to antimycin A.

Ordination of the riffle assemblages (Figure 6) shows clear separation between pretreatment samples and samples taken shortly after treatment. In contrast to treatment 1 at this site, samples taken 4 months later still did not resemble the pretreatment samples, indicating that the assemblage did not yet fully recover. The joint plot function shows that pretreatment assemblages were associated with high ephemeropteran diversity ($r^2 = 0.528$) and that samples taken at 4 months posttreatment were associated with high dipteran diversity ($r^2 = 0.53$).

Control Site

At treatment sites 1 and 2, there was little change in drift, density, or community (as measured by HBI)

TABLE 4.—Results of ANOVA tests examining the effect of antimycin A treatment on changes in invertebrate density and the Hilsenhoff biotic index (HBI) measured at treatment site 2, Fossil Creek, Arizona. The ANOVA types are before–after control–impact (BACI) and BACI paired series (BACIPS). For BACI tests, only the site × date interaction is given. Effects were measured during treatment (immediate) and at 3 weeks (short term) and 4 months (long term) posttreatment.

Sample	Treatment effect	ANOVA type	Response variable	df	*F*	*P*
Drift	Immediate	BACI	Density	1, 57	31.582	<0.001
			HBI	1, 53	3.854	0.055
Riffle benthos	Short term	BACI	Density	1, 39	0.023	0.881
			HBI	1, 39	11.884	0.002
	Long term	BACIPS	Density	1, 7	0.007	0.937
			HBI	1, 7	10.717	0.017
Pool benthos	Short term	BACI	Density	1, 39	0.037	0.848
			HBI	1, 39	1.387	0.248
	Long term	BACIPS	Density	1, 7	2.815	0.144
			HBI	1, 7	1.068	0.341

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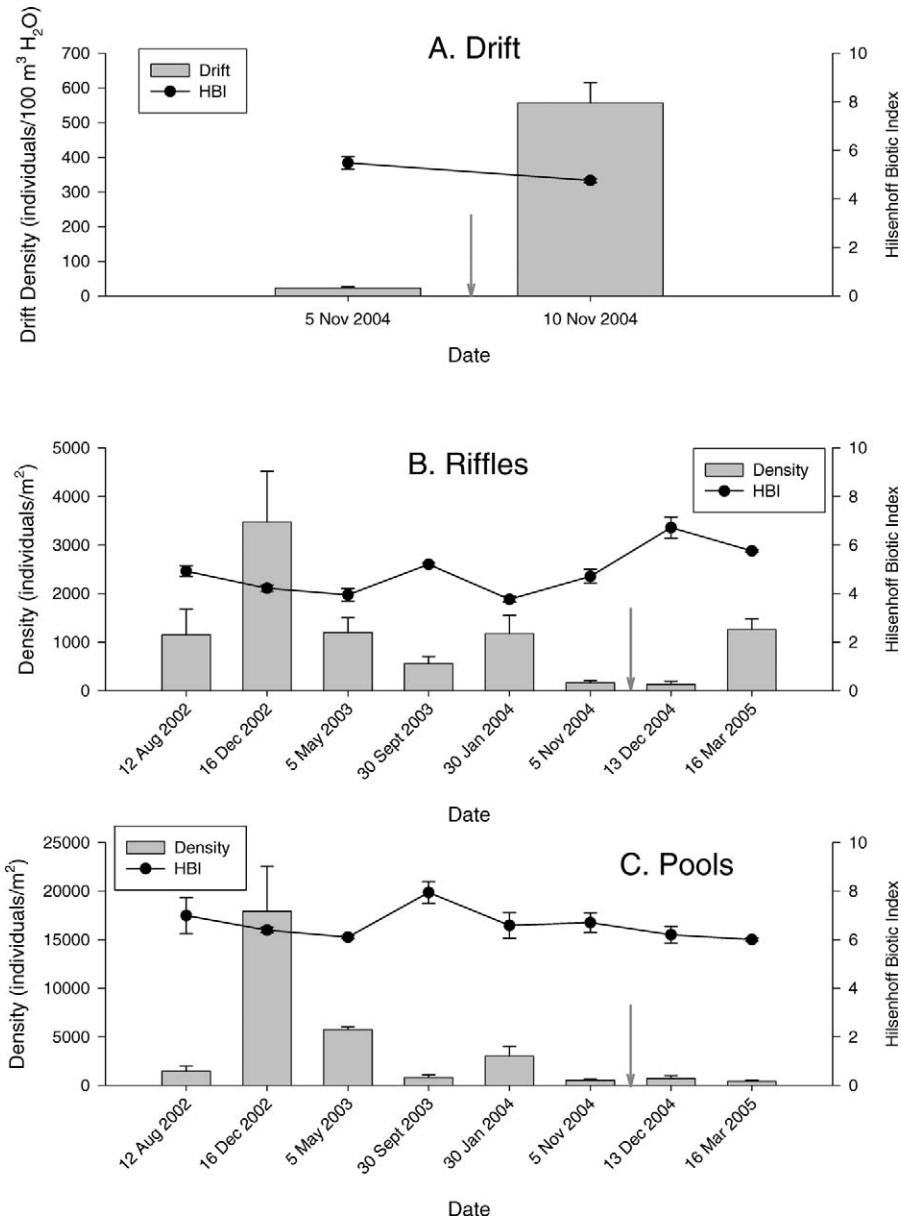

FIGURE 4.—Mean (SE) macroinvertebrate **(A)** drift density, **(B)** benthic density in riffles, and **(C)** benthic density in pools within Fossil Creek, Arizona, treatment site 2 before and after antimycin A application (arrow indicates date of treatment) to remove nonnative fish. The Hilsenhoff biotic index (HBI) is presented in each panel. Drift density was measured during instead of after treatment.

during the treatment periods (Figure 7). Control drift rates (mean) were similar to pretreatment drift rates during the first treatment (site 1: 83.4 indiviuals/100 m^3; control: 73.7 indiviuals/100 m^3) and the second treatment (site 2: 23.3 individuals/100 m^3; control: 17.3 individuals/100 m^3). The decrease in drift between 13 October and 8 November 2004 was probably due to high flows from precipitation events during that period.

Indicator Species Analyses

We found significant losses of invertebrate species in treatment reaches (Table 5). These are species that had still not reappeared after 4 or 5 months, whereas

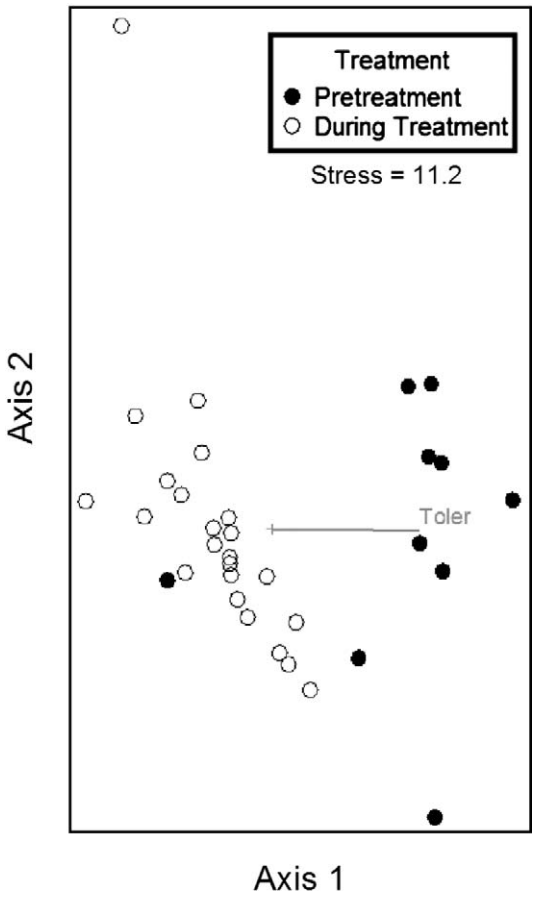

FIGURE 5.—Nonmetric multidimensional scaling ordination of the macroinvertebrate drift assemblage at treatment site 2, Fossil Creek, Arizona, showing relationship among samples taken before antimycin A application (used for nonnative fish removal) and during treatment. The joint plot shows the Hilsenhoff biotic index tolerance variable (toler), which explained more than 30% of the variability ($r^2 > 0.30$).

the control site experienced no loss of species. In treatment site 1, of the nine species that were not found after the treatment, three were significant (Monte Carlo randomization). Treatment site 2 had eight extirpated taxa, three of which were significant. This represented a 7% loss of total diversity at site 1 and a 14% loss at site 2.

Discussion

Our results show that antimycin A at high concentrations can detrimentally affect macroinvertebrates, particularly species composition. Initial mortality rates during treatment were high and dramatically reduced densities. Nevertheless, densities recovered after 5 months at one site and remained only slightly

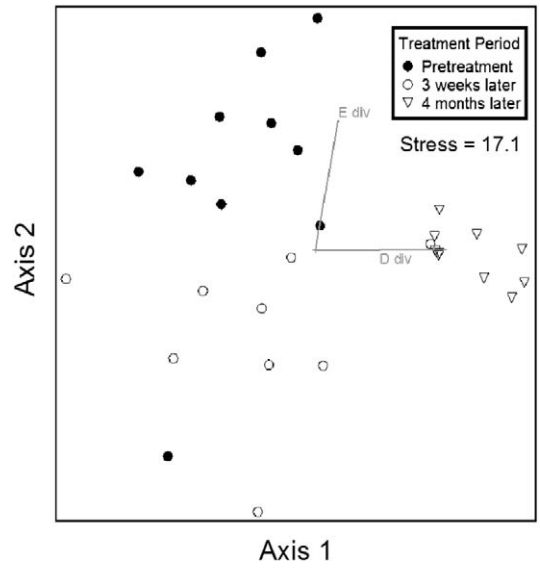

FIGURE 6.—Nonmetric multidimensional scaling ordination of invertebrate assemblages in riffles at treatment site 2, Fossil Creek, Arizona, showing relationship among samples taken before antimycin A application (used for nonnative fish removal) and at 3 weeks and 4 months posttreatment. Joint plots are variables that explained more than 40% of the variability ($r^2 > 0.40$) along axis 1 or 2: dipteran diversity (D div) and ephemeropteran diversity (E div).

depressed at a second site where the highest toxicant concentrations were used. Some species were particularly vulnerable to chemical treatment, failing to recover after 5 months. Short-term reductions in density were more dramatic in pools than in riffles, whereas changes in species composition were more pronounced in riffles than in pools.

The larger effect at treatment site 1 was probably due to the higher concentrations used there and to the higher tolerance of the site 2 assemblage as measured by the HBI. Given additional time, we expect full recovery in the invertebrates in both sites, but this study was limited in duration by the flow restoration, which commenced in June 2005. We will continue to monitor invertebrates particularly to determine recovery of species that were not found after treatment. We anticipate, however, major changes in density and species composition of invertebrates with increased base flow, challenging our ability to detect long-term effects of antimycin A. Future changes in invertebrate densities and assemblages may be a result of restored flows and altered geochemistry rather than recovery from antimycin A effects. Nevertheless, this study is currently one of the most comprehensive evaluations of antimycin A effects on invertebrates.

It is difficult to predict a priori which species may be

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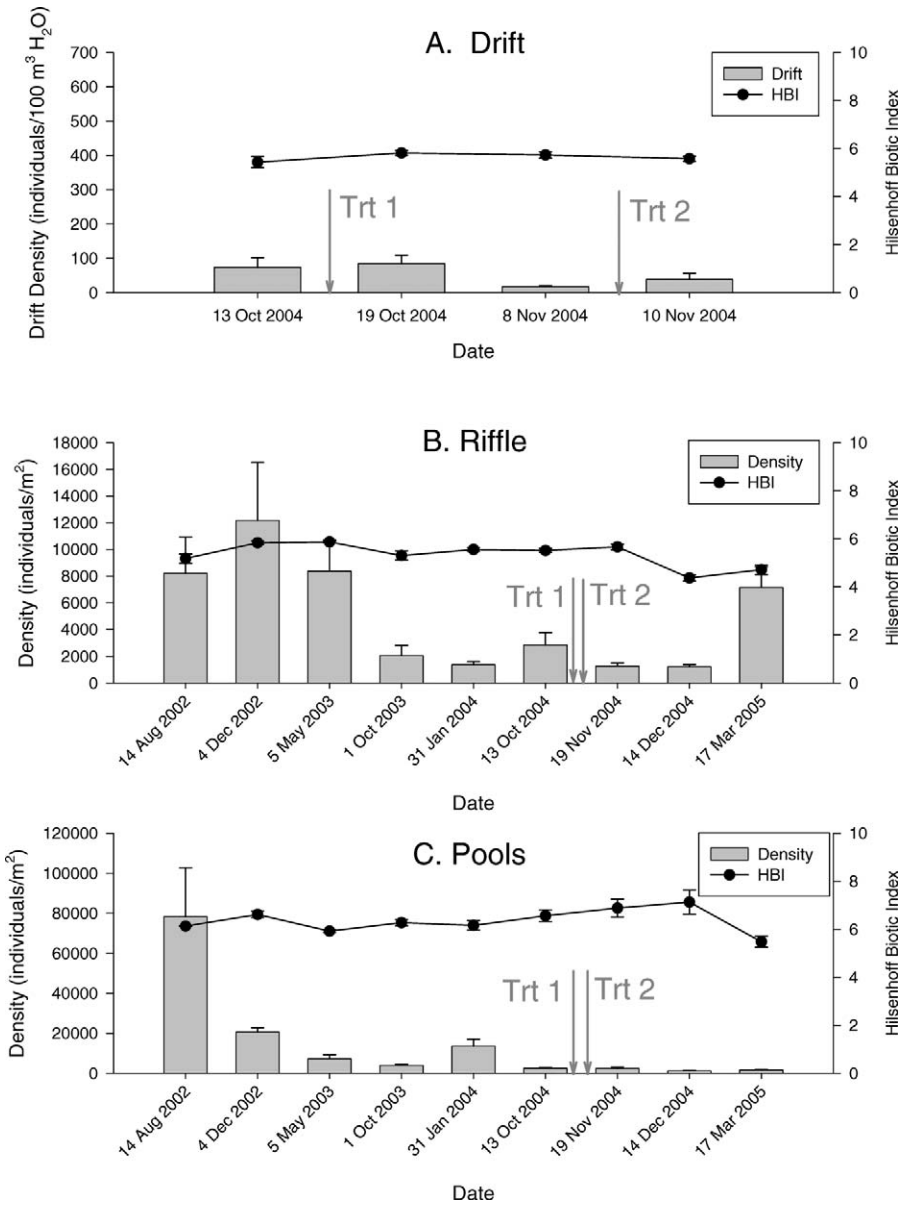

FIGURE 7.—Mean (SE) macroinvertebrate **(A)** drift density, **(B)** benthic density in riffles, and **(C)** benthic density in pools within the Fossil Creek, Arizona, control site before and after antimycin A application to remove nonnative fish in treatment sites 1 and 2 (arrows indicates dates of treatment). The Hilsenhoff biotic index (HBI) is presented in each panel. Drift density was measured during instead of after treatment.

extirpated from the community. Some species were locally extirpated in treatment site 1 but persisted in treatment site 2, and vice versa. No species were eliminated in the control site, suggesting that all extirpations were a result of treatment rather than sampling error. The HBI values were not useful in predicting species losses. The average HBI values for the species lost, 4.3 for treatment site 1 and 4.2 for site 2, were not indicative of sensitive taxa.

Our results are consistent with other studies that showed drastic short-term effects of antimycin A applications of 10–44 μg/L (Jacobi and Deagan 1977; Minckley and Mihalick 1981; Moore et al. 2005). These studies reported recovery of common taxa within

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TABLE 5.—Results of indicator species analysis for control and treatment sites within Fossil Creek, Arizona, showing extirpation of invertebrates after antimycin A application. All listed taxa were absent from any posttreatment sampling. Extirpations significant by Monte Carlo simulation are denoted with an asterisk. The percent affected is the proportion of missing taxa relative to the total number of taxa appearing before treatment (E = Ephemeroptera, D = Diptera, T = Trichoptera, O = Odonata, C = Coleoptera, H = Heteroptera, and L = Lepidoptera).

Control		Treatment site 1		Treatment site 2	
Extirpated taxon	*P*	Extirpated taxon	*P*	Extirpated taxon	*P*
None		*Baetodes* (E)*	***0.002***	Aquatic mites	***0.101***
		Bezzia (D)	0.056	*Chimarra* (T)	0.101
		Chimarra (T)*	0.011	*Corydalus* (M)*	0.031
		Haeterina (O)	0.248		0.098
		Lutrochus (C)	0.252	*Hydropsyche* (T)*	0.001
		Metrichia (T)	0.255	*Lutrochus* (C)*	0.029
		Rhagovelia (H)*	0.003	*Petrophila* (L)	0.325
		Tinodes (T)	0.056	*Tricorythodes* (E)	0.331
		Tricorythodes (E)	0.255		
Total significant	0	Total significant	3	Total significant	3
Percent affected	0	Percent affected	7	Percent affected	14

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1–3 years. Moore et al. (2005) saw declines in species of Ephemeroptera, Plecoptera, and Trichoptera in immediate response to antimycin A but observed rapid recovery within 4 months; they cautioned, however, that substantial variation was present because of sampling error by multiple collectors. Species responses were not reported by Jacobi and Deagan (1977), whereas Minckley and Mihalick (1981) reported that six species were still absent after 3 years, possibly due to sampling error or floods. Our analysis, however, revealed that the absences of some species in Fossil Creek are probably not due to sampling error or natural disturbance but are due to the chemical treatment. In contrast to our results, other researchers report no negative effect of antimycin A on macroinvertebrates, probably because their studies involved lower concentrations (10 μg/L; Gilderhus et al. 1969; Morrison 1979; Minckley and Mihalick 1981; Cerreto 2004). Some of these studies were limited to artificial pools and few to no controls were used (Snow 1974; Houf and Campbell 1977).

Interestingly, some government studies have concluded that antimycin A is safe for invertebrates while documenting mortality rates of 50–99% for certain taxa (Walker et al. 1964; Gilderhus et al. 1969). Nevertheless, it was concluded that antimycin A was "largely specific to fish and causes no harm to most of the other aquatic animals" (Gilderhus et al. 1969: page 20) and that there were "no grossly toxic effects" (Walker et al. 1964: page 14).

Recovery in Fossil Creek was probably facilitated by the location and timing of the project. First, there was an upstream site that was untreated. Because invertebrate colonization is largely from upstream sources, the presence of a colonizing source nearby in the same watershed almost certainly increased recovery rates. Pretreatment surveys by AZGFD and Northern Arizona University had identified the specific upstream extent of nonnative fish, smartly eliminating the need to treat these areas. Second, AZGFD timed the project for the late fall, when many Arizona streams experience heavy rain and flash floods. Desert aquatic invertebrates are adapted to such events, finding refuge in the hyporheic zone or progressing to a different life stage (i.e., terrestrial adult). This was evident in the control samples that showed low densities during the treatment period relative to other sampling periods. Although trying to implement a large-scale project during inclement fall weather was not easy, placement of artificial disturbance in the background of natural disturbance may have minimized the overall effects to the biota.

The use of antimycin A to eradicate nonnative fish increased in the 1990s as managers confronted the difficult issues of conserving native fish; the increasing trend in antimycin A use will probably continue (Finlayson et al. 2002). Our results (with the qualification of the extraordinary concentrations used) and others indicate that antimycin A can kill macroinvertebrates and that some species may not recover. The dearth of peer-reviewed studies evaluating the effects of antimycin A on macroinvertebrates argues for including comprehensive monitoring programs as components of chemical treatment. This will help scientists and managers build a database to assess the effects of antimycin A under different field conditions. In Fossil Creek, macroinvertebrates were scarcely mentioned in an environmental assessment (USFS 2003) and were not included in the subsequent monitoring program, in part because of the commonly

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held belief that antimycin A does not harm macroinvertebrates. As with all management actions, the concerned parties must carefully weigh the risks and benefits, allowing stakeholders to decide whether the risk to aquatic invertebrates is justifiable for the continued health of native fisheries.

Four general observations that emerged from this study can be applied to such proposed projects. First, if there are listed or endemic macroinvertebrate taxa in the study site, then antimycin A treatment should have provisions for protecting these taxa. In Fossil Creek, there are two species of special concern. Pretreatment surveys revealed that both species were concentrated above the diversion dam in an area that would not be treated. In projects where species of special concern are not naturally protected, the protocol should include a plan for salvaging individuals before treatment or for ensuring posttreatment recolonization. Second, in projects where native fish are reintroduced into the river from other sites or captive populations, it may be prudent to use a macroinvertebrate recovery period of at least 6 months before reintroducing fish species that feed on macroinvertebrates. Third, in situ bioassays can help predict site-specific effects. In the Fossil Creek project, we worked with AZGFD personnel while they determined the necessary antimycin A concentrations. To do this, we measured drift in small test reaches and were able to advise that high mortalities of aquatic invertebrates would probably occur upon full treatment. Although this method cannot predict long-term impacts, it does give managers the information needed to judge the risks and benefits involved. Multiple bioassays at a variety of concentrations can also help determine the best choice: a concentration low enough to minimize effects on aquatic invertebrates but high enough to kill all fish. When the required amount of antimycin A will unavoidably cause large kills of invertebrates, an alternative piscicide may be preferable, such as rotenone, which is more accessible and cost effective. This suggestion is based on the premise that one of the main advantages of antimycin A over rotenone is lower aquatic invertebrate mortality. Another alternative is use of multiple exposures (e.g., 2 d of treatments at lower concentrations) instead of a 1-d treatment at a high concentration. Fourth, when possible, projects should be timed to coincide with natural disturbance regimes or with a period when most aquatic insects are in terrestrial stages.

By drawing on a large pretreatment database, we were able to view changes caused by chemical treatment within the context of seasonal and annual variation. Results from this study will inform future projects in which managers are considering use of piscicides. We also urge continued study of nontarget organisms at a range of treatment concentrations to help establish a database to improve prediction of antimycin A effects.

Addendum

During the manuscript submission and review process, we have continued to monitor the invertebrate assemblages of Fossil Creek in 2005 and 2006. Although large-scale changes have occurred due to flow restoration, several extirpated invertebrates have returned to the treatment reaches. By August 2006, five taxa had recolonized treatment site 1 (*Baetodes*, *Bezzia*, *Metrichia*, *Rhagovelia*, and *Tinodes*, whereas four taxa had not returned (*Chimarra*, *Hetaerina*, *Lutrochus,* and *Tricorythodes*). At treatment site 2, all except *Chimarra* (absent in 2005 and 2006) had returned. However, the absence of any of these species may now be due to the restoration of flows and changing amounts of travertine and not due to continued effects of antimycin A.

Acknowledgments

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