AQUATIC CONSERVATION BIOLOGY IN ARID ECOSYSTEMS

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ABSTRACT

AQUATIC CONSERVATION BIOLOGY IN ARID ECOSYSTEMS

Eric C. Dinger

Aquatic conservation in arid ecosystems is a pressing concern for the public and land managers in the Southwest. This study focuses on aquatic conservation issues in 2 sites – 1 international site, Cuatro Ciéneags, México and 1 Arizona site, Fossil Creek which was the focus of a collaborative, multi-faceted stream restoration project.

In Cuatro Ciénegas, we conducted an experiment manipulating fish access to stromatolites. We manipulated 2 fish species that occur with stromatolites, the polymorphic *Herichthys minckleyi*, and the pupfish *Cyprinidon bifasciatus*. We used a trophic cascade index as an indicator of cascade strength, and only molariform morphs were responsible for a trophic cascade, reducing snail densities so that stromatolite algal biomass was positive. The papilliform morph treatments, in contrast, allowed snail densities to increase, resulting in stromatolite algae declines indicating loss of stromatolite formation. Our results show that modern stromatolite formation requires the presence of a specific keystone morph of an endemic threatened cichlid. Our results are also consistent with the hypothesis that metazoan grazing could have been responsible for ancient stromatolite declines, and modern stromatolites should be studied in the context of the entire ecosystem.

Restoring native fish to freshwater habitats often requires removal of exotic fish using chemicals such as Antimycin A. We studied the immediate and lingering effects of Antimycin A on macroinvertebrates during a fish renovation project in Fossil Creek,

Arizona. We employed before-after-control-impact designs to measure the effects of Antimycin A (at 54 μg/L and 100 μg/L) on macroinvertebrate drift, densities and species composition. At the highest dose (100 μg/L) Antimycin A increased drift five fold and immediately decreased invertebrate standing stocks in pools and riffles. Although Antimycin A effects were mostly short-term, several species were extirpated.

We studied the short-term effects of restoration of flows to Fossil Creek after 100+ years of flow diversion. Invertebrate density and diversity was unaffected, but there was a rapid response in species composition to flow restoration at restored sites. Downstream sites shifted as a response to flow, but long-term effects will likely be the result of changing geomorphology associated with changing travertine deposition.

Acknowledgements

Dissertation projects do not occur by themselves or through the work of one individual. I received a large amount of help from a wide variety of people – so many I cannot hope to list them all here.

First and foremost, thanks to my family (especially my parents) who instilled my love of the natural world at an early age. Thanks also to my high school biology teachers who started me down this path.

I am also indebted to the entire graduate community at Northern Arizona University, especially Bill and Anne Stanely, Rusty and Bianca Perla, Matt and Heidi O'Neill, Brook and Lisa Swanson – these people were my role models for my entire graduate career. I can only hope to live to be as good as they are.

Thanks to all the people who provided various assistance throughout: Joe Shannon, Bruce Hungate, Alice Gibb, Mike Kearsely, Eric North, Kevin Wilson and Allen Haden. They all provided me with advice or field assistance during my Ph.D. studies, as well as my Masters degree.

Special thanks to Dr. Rick Doucett – who help me in so many ways, it's impossible to remember them all. He single-handedly helped me remember my joy in climbing, running, and beer drinking. And then became a willing partner in all of these pursuits.

As a person who disdains paperwork, thanks to the entire Biology office staff who were very accommodating as I dealt with filing various papers! And to Dr. Ron Markle who (usually) was very patient with my questions.

My committee was also incredibly patient with me – from my late start in even having a committee to the final rush to finish my degree. Phil Service, Tad Theimer and Rod Parnell were the ideal committee to see me through this.

Dr. Jane Marks was my collaborator through all of this – She helped me learn how to be a good scientist, educator, professional and human being. It has been a joy working with Jane from the start of both of our careers – she was the prefect advisor to me.

Despite this help, I don't think it would have finished without the help of one last person – my loving wife Nikki. In so many ways, this is not my dissertation, it is our dissertation! Her guidance, support and encouragement provided me the light in the many dark tunnels during my education.

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To my wife, Nikki

Preface

This dissertation is written in journal format, with 4 total chapters. Each chapter has been written as a stand alone submission for publication in selected scientific journals. Because studies like this rarely occur without help or guidance, all of these chapters received help of co-authors. In this, I have retained the original writing style for publication – using "we" instead of "I". Furthermore, I have included the names of my co-authors at the start of each chapter.

Chapter 1 is a short paper format, and offers a brief explanation and examination of my studies on stromatolites in Cuatro Ciénegas, Coahuila, México. It was accepted for review but declined for publication as a *Science Brevia* manuscript. In the future, it may be submitted as a brief in another journal.

Chapter 2 is a more formal, extended analysis of my work on stromatolites. The study site and questions are similar to those presented in chapter 1, but is a more thorough examination. It is intended for publication in the journal *Ecology*.

Chapter 3 and 4 focus on my studies on the conservation biology surrounding the restoration of Fossil Creek. Chapter 3 studies the effects of using a high dosage of a fish piscicide, antimycin A on the aquatic invertebrate assemblages. It has been accepted for publication in the *North American Journal of Fisheries Management*, pending revisions.

Chapter 4 is about the response of the aquatic macroinvertebrates to flow restoration after 100+ years of flow diversion. The time span of my education and the realities of dam decommissioning timelines have given me the opportunity for a large, pre-restoration dataset, with the side-effect of limiting my observations to short-term

responses (~15 months). It will be submitted to a freshwater ecology journal, such as the *Journal of the North American Benthological Society.*

Predatory fish sustain modern stromatolites

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Abstract:

Stromatolites, a dominant life form in the Precambrian Era, declined in diversity and abundance in the fossil record during the Cambrian transition – although rare, modern living examples are scattered across the globe. We show that trophic cascades, where predators control herbivores, releasing primary producers from grazing pressure, are instrumental in the persistence of stromatolites in Cuatro Ciénegas, Mexico. Our results indicate that a single morphotype of a threatened, endemic fish is critical for maintaining this rare life form. The observation that high densities of invertebrates halt stromatolite growth is consistent with the hypothesis that grazing invertebrates contributed to their demise.

Text:

Stromatolites, a dominant life form in the Precambrian Era, declined in diversity and abundance in the fossil record during the Cambrian transition – although rare, modern living examples are scattered across the globe. Because algal photosynthesis facilitates the precipitation of calcium carbonate that causes stromatolite growth, the radiation of metazoan grazers could explain the precipitous decline of stromatolites ~570 mya, as grazers consumed the essential algal biofilm (*1*). The concurrence of metazoan radiation and stromatolite decline in the fossil record, along with the observations of modern stromatolites in locations where extreme environmental conditions limit grazers support this hypothesis (*2*). Further evidence comes from observations that increasing grazing pressure reduces calcium carbonate precipitation (*3*). The occurrence of metazoan grazers with modern stromatolites in more benign habitats argues against strong grazer control, yet, to our knowledge, the influence of higher trophic levels on stromatolites has never been investigated. Here, we show that trophic cascades, where predators control herbivores, releasing primary producers from grazing pressure, are instrumental in the persistence of stromatolites in Cuatro Ciénegas, Mexico, one of the few sites in the world with abundant freshwater stromatolites. Specifically our results indicate that a single morphotype of a threatened, endemic fish is critical for maintaining this rare life form.

We examined the influence of trophic interactions on stromatolites in the Río Mesquites of Cuatro Ciénegas, Mexico, which houses numerous endemic fish and snails and is recognized globally as a biodiversity hotspot. Our year-long field experiment occurred in

an area of the river with fields of stromatolites typical of the basin. We placed stromatolites in mesh cages in combination with various species of fish, focusing on the polymorphic cichlid, *Herichthys minckleyi* (*4*). This species is widespread in the basin and occurs in two main morphotypes – papilliform morphs that eat soft-bodied invertebrates and algae, and molariform morphs with robust pharyngeal dentition capable of crushing snails (*5*), including the two endemic snails that are dominant grazers on stromatolites, *Mexithauma quadripallium* and *Nymphophilus minckleyi*. We compared treatments with the molariform present and absent in combination with the papilliform morph and other common fish. When molariforms were present, regardless of the composition of other fish, snail densities matched ambient stromatolites. In contrast treatments excluding molariforms had approximately 300% as many snails (student's t = 4.91, $P < 0.0001$, Fig. 1A). Concurrent with the increase in snails, the algal biomass was reduced by over 40% (student's $t = 4.17$, $P = 0.0002$, Fig. 1A). Thus, molariform fish controlled snail densities, releasing stromatolites from grazing pressure. When molariforms were removed algal growth diminished such that the stromatolites appeared as non-living rocks. SEM micrographs demonstrate that stromatolites with increased snail densities (Fig. 1B) are noticeably lacking the algal biofilms typical of ambient stromatolites (Fig. 1C). Because calcium carbonate accretion rates of natural Río Mesquites stromatolites barely exceed erosive forces (*3*), any decrease in the photosynthesis driven calcium carbonate deposition will halt stromatolite growth.

These results show that trophic interactions can be a key component for maintaining stromatolite growth. In Cuatro Ciénegas, preservation of this ancient life form requires

the persistence of a specific morphotype of an endemic fish that is threatened by exotic species and water extraction (*6*). The observation that high densities of invertebrates halt stromatolite growth is consistent with the hypothesis that grazing invertebrates contributed to their demise. In Cuatro Ciénegas, stromatolites rely on the presence of predators which do not appear in the fossil record until the Silurian Period ~ 438 mya. The absence of predators during early radiation of grazing metazoans could explain why stromatolites were so susceptible to grazing pressure during their decline.

References and Notes

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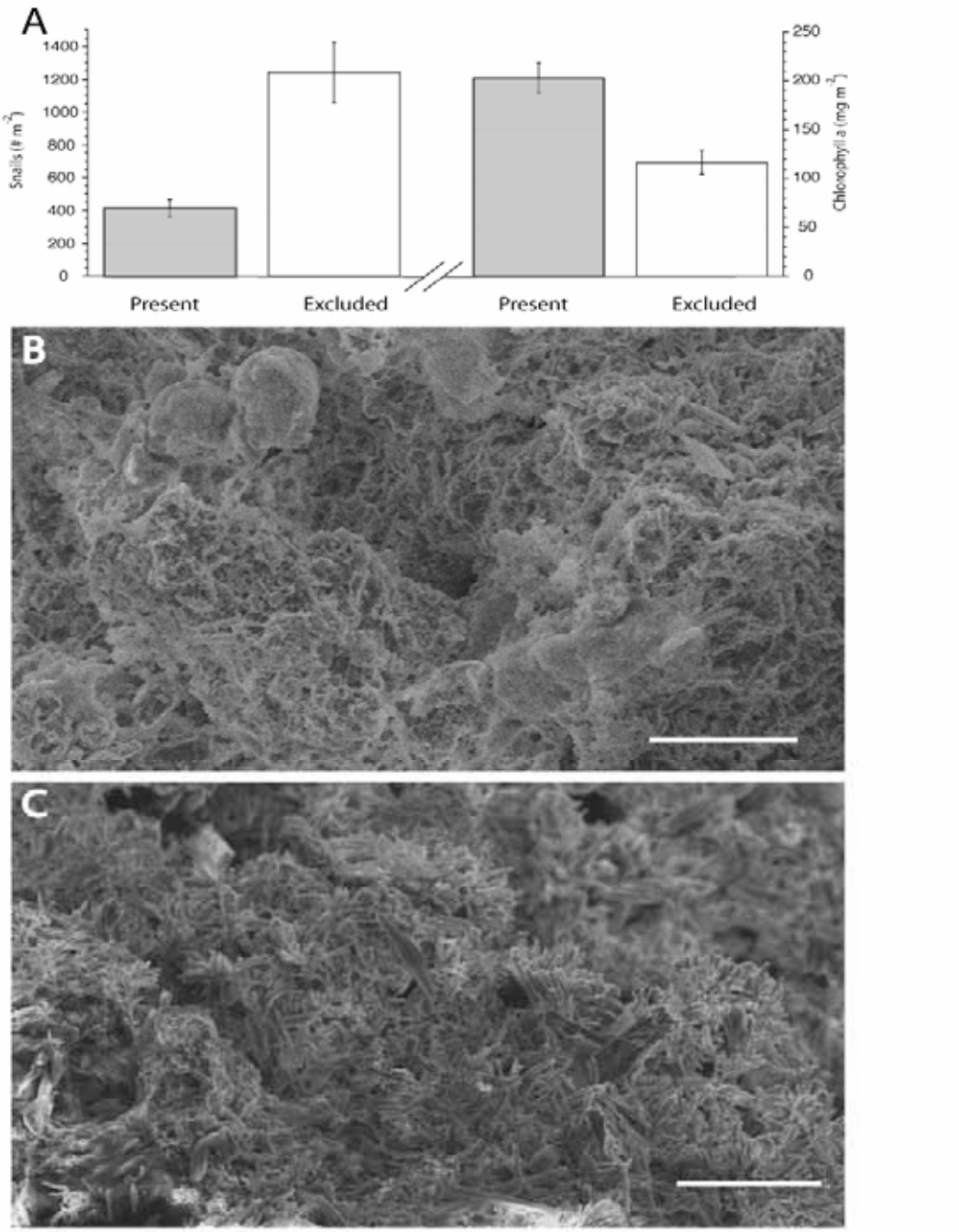

Fig. 1. (A) Trophic cascade promotes stromatolite growth. High snail abundance and low algal biomass (chlorophyll a) when predatory molariform fish are excluded (white) compared to treatments where they are present (gray). Values are means ± SEM. (B) Scanning electron micrograph of a typical stromatolite in the absence of molariforms and subject to high snail densities; very few algal cells are evident. (C) Scanning electron micrograph of a typical stromatolite accessible to predatory molariform fish showing a dense mat of diatoms and filamentous algae. Scale bars, 100 µm.

Materials and Methods

We conducted the experiment in the outflow of Mojarral Este which forms the Río Mesquites (26° 55' N, 102° 07' W) in the Area Protegida de Flora y Fauna, Cuatro Ciénegas, Coahuila, México. High densities of oncoid stromatolites (5.8 ± 0.76 per m^2) occur in the stream channel.

The experiment was started in April 2003 in 40 1 meter squared caged exclosures/enclosures constructed of plastic aquaculture mesh (6.35 mm pore size) and PVC frames. Cages were stocked with 5 oncoid stromatolites (mean diameter 15 cm). Ten of the 40 cages were open on 1 side to serve as cage controls, exposing the stromatolites to the natural community. Ten cages were randomly stocked with *Herichthys minckleyi* papilliform morphs, 10 were stocked with *H. minckleyi* molariform morphs, and the last 10 excluded both morphs of *H. minckleyi*. Morphotypes were identified prior to stocking using an otoscope.

In May 2004, we collected 1 random stromatolite from each cage. On each stromatolite, we took a 17.8 mm^2 core sample for Chlorophyll *a* analysis. Cores were placed in aluminum foil and frozen for transport. Chlorophyll *a* was measured following standard protocols (*S1*) on a Perkin-Elmer Coleman 124 Spectrophotometer, and calculated to Chlorophyll *a* amount. In addition to caged stromatolites, we also sampled 10 ambient stromatolites.

To collect invertebrates, we created an anaerobic environment to cause invertebrates to leave the stromatolite (*S2*). Stromatolites were placed in water filled 5-gallon plastic buckets and CO_2 was bubbled into the water to drive out oxygen. We monitored the progress using a Hydrolab minisonde water probe. Once the dissolved

oxygen content was less than 0.50 mg/l, stromatolites were left for 10 minutes while invertebrates emigrated from the stromatolite. The stromatolite was removed and scanned for additional invertebrates clinging to the exterior. The water with invertebrates was strained through 1mm aquarium mesh nets. Invertebrates were identified and counted using Leica dissecting scopes. Invertebrate densities were standardized to individuals per m^2 using digital photograph measurements to calculate stromatolite size.

Due to fish escape, vandalism and other acts of nature, 10 replicates were lost during the experiment. Nine cage controls, 4 molariform, 8 papilliform, and 9 *H. minckleyi* exclusions survived.

For assessing effects of the molariform morph, we grouped each treatment into either 1) Molariform present or 2) Molariform absent. The " Molariform present" group included molariform enclosures, cage controls, and ambient stromatolites. , The "Molariforms absent" group included the papilliform enclosures, and the *H. minckleyi* exclosures. Data were analyzed using student's *t* test using JMP IN 4 software package (*S3*).

References for methods and materials

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S2. Thanks to the late W.L. Minckley for suggesting this collection technique.

S3. JMPin version 4.0.2 SAS Institute Inc. (2000).

S4. We thank the Cuatro Ciénegas Park staff (especially Arturo Contreras-Balderas); B. Winsborough, D. Hendrickson, M. Sellers and many volunteers. Supported by TNC, NSF, EPA, NAU, and the PSA.

Modern Stromatolites in their ecosystem: Is modern stromatolite growth maintained by higher trophic levels?

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Abstract

We conducted a year long experiment manipulating fish access to stromatolites, a rare algal life-form dominant in the Precambrian. Modern stromatolites now only occur in isolated ecosystems, and it was hypothesized that metazoan grazing caused stromatolite decline in the Cambrian Transition. However, occurrence of diverse and abundant invertebrate grazers with modern stromatolites in Cuatro Ciénegas, México suggested that evolution of grazers was unrelated to ancient stromatolite declines. We tested the hypothesis that a third trophic level, vertebrate predation, controlled the metazoan grazer densities, minimizing grazer effects so that stromatolites could persist. We manipulated 2 fish species that occur with stromatolites, the polymorphic *Herichthys minckleyi*, and the pupfish *Cyprinidon bifasciatus*. We hypothesized that only 1 morph of *H. minckleyi*, with molariform dentition capable of consuming the dominant grazer, native Hydrobiidae snails, would control snails, allowing stromatolite growth. We used a trophic cascade index as an indicator of cascade strength, and only molariform morphs were responsible for a trophic cascade, reducing snail densities so that stromatolite algal biomass was positive. The papilliform morph treatments, in contrast, allowed snail densities to increase, resulting in stromatolite algae declines indicating loss of stromatolite formation. Pupfish, however, caused declines in both snail and algal biomass presumably due to direct competition with snails, coupled with pupfish herbivory on the stromatolites. Our results show that modern stromatolite formation requires the presence of a specific keystone morph, of an endemic threatened cichlid. Our results are also consistent with the hypothesis that metazoan grazing could have been

responsible for ancient stromatolite declines, and modern stromatolites should be studied in the context of the entire ecosystem.

Introduction

Stromatolites, an ancient, once dominant algal life form that causes mineral deposits to form a rock like structure, declined in diversity and abundance in the Precambrian (~ 570 mya) concurrent with the rise of metazoans (Awramik 1971). This led to the hypothesis that metazoans, through grazing and bioturbation, caused the demise of stromatolites (as summarized by Gebelein 1976, but also see Garrett 1970, Awramik 1971, Walter and Heys 1985). This hypothesis is indirectly supported by the occurrence of most extant stromatolites in "extreme" environments, which have low densities of grazers (e.g. Monty and Hardie 1976, Playford and Cockbain 1976). The relationship between invertebrates and stromatolites, however, is not fully understood (Farmer 1992) with recent studies pointing to the co-occurrence of stromatolite-like microbial mats and grazers in some habitats (e.g. Laguna Guerrero Negro, Baja California Sur, México, Cuatro Ciénegas, Coahuila, México) suggesting that the decline of stromatolites was unrelated to metazoan appearance. Alternative hypotheses for stromatolite declines postulate that stromatolites were out competed for space by other algal forms (Pratt 1982) or that changes in seawater chemistry created unfavorable conditions for stromatolites (Grotzinger 1990).

Studies on the effects of grazers on stromatolites have yielded mixed results. Some studies have rejected the grazer hypothesis by noting the inability of grazers to completely limit or structure microbial mats and stromatolites (Farmer 1992, Elser et al. 2005). In contrast, other studies have suggested strong grazer control of stromatolite

algal assemblages and growth rates (Winsborough 1990, Dinger 2001, Garcia-Pichel et al. 2004, Dinger et al. 2006). Most of these studies are either based on short-term observations, or were simplified studies focusing on only one aspects of the community. We maintain that a better test of how trophic interactions affect stromatolites requires longer field studies where entire grazer assemblages can be manipulated.

Here we describe a yearlong field experiment testing how fish affect stromatolites and invertebrate communities in Cuatro Ciénegas, México one of the few sites worldwide with freshwater stromatolites. Despite the challenges of extrapolating from a modern food web with fish and invertebrates to a Precambrian food web, where vertebrates had yet to evolve, this study elucidates the potential for higher trophic levels to control stromatolite growth. Specifically, we manipulated the accessibility of three dominant fish morphs/species with different mouth parts and feeding strategies to compare the direct and indirect effects of fish on stromatolites and associated invertebrate communities. We predicted that the molariform morphs of the endemic cichlid (*Herichthys minckleyi*), which can crush and consume snails (Husley et al. 2006), would have a direct negative effect on snails and an indirect positive effect on stromatolites via a trophic cascade, illustrating the potential for grazers to reduce stromatolite growth as suggested by Garrett (1970) and Awramik (1971). In contrast, we expected that omnivorous pupfish, which eat primarily algae and detritus, would have direct negative effects on stromatolites. We expected that the papilliform morph of the dominant cichlid, which consumes detritus, algae, and soft-bodied invertebrates, would have a direct negative effect on stromatolites that was slightly tempered by their ability to reduce grazers. We compared the interaction strengths of the three fish using a trophic index

described by Osenberg et al. (1997) to determine which species have the strongest effects on structuring stromatolites and their associated invertebrate assemblages. This index has been used as a standardized metric for measuring both the direct effects of predators on herbivores and the indirect effects of predators or trophic cascade strength, on primary producers (Shurin et al. 2002, Borer et al. 2005).

Methods

Study site

Cuatro Ciénegas, Coahuila, México in the Chihuahuan Desert, is one of the few places globally with freshwater stromatolites. We conducted our studies in the outflow of Mojarral Este, which forms the Río Mesquites (26° 55' N, 102° 07' W) in the Area Protegida de Flora y Fauna. The basin of Cuatro Ciénegas was declared a protected area in 1994 by the Mexican government due to a variety of ongoing threats to the biological diversity of the area (Minckley 1992). The high amount of endemism in the basin has been well described in the literature (e.g. Cole 1984, Hershler 1984), as have the habitats of the basin (esp. Minckley 1969).

The water chemistry of Río Mesquites is dominated by Ca^{2+} and SO_4^{2-}. High densities of oncoid stromatolites (5.8 ± 0.76 per m^2), shaped like oblong spheres with diameters ranging from 2-30 cm are found in the middle of the channel. These stromatolites of Cuatro Ciénegas are laminated, benthic, microbial calcium carbonate deposits caused by biological activity, where the microbial components incorporate carbonate into their extracellular material. Algal components of the stromatolites are dominated by the green alga, *Gongrosira calcifera* Krieger, *Cyanostylon microcystoides;* the Cyanobacteria *Homeothrix balearica* Bornet and Flahault, *Schizothrix lacustris* A.

Brown; and the diatoms *Eunotia sp., Amphora katii* Selva, *Epithemia argus* Kütz, and *Gomphonema intricatum* Ehr. (Winsborough 1990). For more details of the study site see Dinger et al. (2006).

These stromatolites harbor a diverse invertebrate assemblage dominated by two endemic snails, *Nymphophilus minckleyi* and *Mexithauma quadripalium*, (Hydrobiidae) which constitute roughly 45 percent of the invertebrate biomass, although Chironomidae midges (Diptera) and *Hyalella* sp. (Amphipoda) are often numerically dominant (Dinger et al. 2005). There are three dominant fish in our study site: 1) the molariform morph of the endemic cichlid *Herichthys minckleyi* which has dentition capable of crushing snails (Hulsey et al. 2005), 2) a papilliform dentition morph that rarely consumes snails but eats algae, detritus, and soft bodied invertebrates (Hulsey et al. 2005) and 3) the small pupfish *Cyprinidon bifasciatus*, which feeds on algae, detritus and soft-bodied invertebrates.

Experimental design

We ran two experiments side by side for 380 days, from 20 April 2003 to 10 May 2004. One experiment tested the effects of different morphs of *H. minckleyi* on stromatolites, and the other tested the effects of *C. bifasciatus* on stromatolite assemblages.

Experiment 1 – Herichthys minckleyi

We used 1 m^2 cages made of PVC framing wrapped in 6.35 mm plastic aquaculture mesh to exclude/include different morphs of *H. minckleyi*. Cages were 1 m tall, allowing the top of the cages to extrude from the water so that no tops were necessary and fish could not jump into or out of cages. Previous attempts at cages without bottoms proved ineffective at maintaining desired fish densities, necessitating

cage bottoms of 1 mm hardware mesh, with approximately 50 haphazard 3 cm slits to facilitate invertebrate migration from the sediment to the stromatolites.

Every treatment type was replicated 10 times. One treatment was stocked with a single molariform morph, one was stocked with a single papilliform morph, and one was not stocked with *H. minckleyi*. Additionally, one treatment was constructed identically to the other cages, but with one full side open to the environment to act as a cage control. The cage control served to mimic the possible cage artifacts of reduced water velocity and sunlight. The aquaculture mesh allowed the smaller pupfish *C. bifasciatus* to enter and exit the cage unimpeded. This allowed us to assess the effects of *H. minckleyi* morphs in the context of the rest of the ecosystem.

Experiment 2 – Cyprinidon bifasciatus

To test the effects of the pupfish, *C. bifasciatus*, on stromatolites, we constructed 1 m^2 cages made of PVC framing wrapped in 1mm fiberglass hardware mesh. Other than using a smaller mesh size, cages were identical to those used in experiment 1. Cages were assigned to one of three treatments (n = 10 for each): 1) *C. bifasciatus* only (initially stocked with 10 pupfish each), 2) No fish, and 3) Cage controls, identical to experimental cages, but with one side open to the ambient stream, allowing fish to enter and exit the cage control.

Field and lab protocols

At harvest time, a random stromatolite was collected from each cage. Stromatolites were slowly and carefully lifted out of the water, and a 1mm mesh aquarium net was used to retain snails or other invertebrates dislodged during removal. Stromatolites were digitally photographed and measured for height. Mini-cores made of

aluminum tubing (0.71 cm^2) were used to take samples for Chlorophyll pigment analyses. These cores were driven through the top surface of the living biofilm of the stromatolite to lower lithified layers. The cores were then placed on ice for later analysis in the lab. Stromatolites and any dislodged invertebrates were then placed in 5-gal buckets.

Invertebrates were removed by creating a low oxygen environment in each bucket, a technique recommended by the late W.L. Minckley. Stromatolites were immersed in 20 l of water, and dissolved CO_2 was aerated (~ 0.34 m^3/hr) until the O_2 level dropped below 0.5 mg l^{-1} (monitored with a Hydrolab Minisonde 4 oxygen sensor). When the O_2 level dropped below 0.5 mg l^{-1}, the CO_2 addition was halted, and kept in stasis for 10 additional minutes. Stromatolites were then removed from the bucket and visually inspected for any invertebrates remaining on the stromatolite surface. Special attention was given to the rugulose crevices of the stromatolites, where narcotized invertebrates may have been stuck. Any invertebrates encountered were added to the floating, narcotized invertebrates in the bucket. The contents of the bucket were filtered through a 250 μm mesh net, and preserved in 95% ethanol. Each stromatolite was then returned to the stream, barren of invertebrates, but otherwise undisturbed. Other methods of removing invertebrates, such as simply picking invertebrates off with forceps would overlook many invertebrates. This method allowed us to collect invertebrates while protecting these rare, protected life forms.

Invertebrates were identified and enumerated using a dissecting microscope in the lab. Identifications of non-gastropod invertebrates were determined using North American invertebrate keys (Pennak 1989, Thorp and Covich 1991, Merritt and Cummins 1996). Snails were identified using pictures (Taylor 1966, Hershler 1985).

Invertebrate densities were standardized to individuals per m^2, based on the size of the stromatolite. Stromatolite height (measured in the field) was averaged with width and length (measured from digital photographs) to determine an average radius, which was then used in the standard formula for the surface area of a sphere ($S = 4\pi r^2$).

Chlorophyll pigment analysis was used as a surrogate for biomass. Other methods, such as Ash-Free-Dry-Mass were not used because of difficulties in separating combustible carbon and water trapped in the lower layers of calcium carbonate from the upper organic algal carbon as well as potentially older, organic carbon trapped prior to the experimental period. Chlorophyll was extracted in acetone following standard methods (Greenberg et al. 1992). Chlorophyll concentration was measured with a Perkin-Elmer Coleman 124 Spectrophotometer. We used a multi-spectral analysis to simultaneously determine Chlorophyll *a, b,* and *c* concentrations (Greenberg et al. 1992). Chlorophyll concentrations were then converted to chlorophyll biomass per m^2.

Statistical analyses

Effects of fish manipulation were analyzed using Analysis of Variance (ANOVA) routine in the statistical computer package JMPin (version 4.02). Post-hoc pair wise differences were measured using Tukey's HSD test. Statistical significance was set at a *p*-value of 0.05. Least squares regression was used to investigate the relationship between algal biomass and invertebrate densities.

We used the log ratio of plant biomass (Chl *a* mg m^{-2}) or herbivore density (snails m^{-2}) in the presence versus absence of pupfish and cichlid morphs to assess the trophic cascade strength (e.g. Osenberg et al. 1997). Reasons for using the log ratio include 1) clear biological meaning (proportional change in the response variables), 2) it has strong

statistical properties (Shurin et al. 2002) plus 3) it allows comparisons to other published meta-analyses of cascade strength (Shurin et al. 2002, Borer et al. 2005). Positive log ratios on algae can be interpreted as a trophic cascade, whereas a negative log ratio indicates predation pressure (i.e. decreases in algal biomass or snail densities). Additionally, we used the log ratio to examine the effect of the entire fish assemblage (molariform, papilliform, and pupfish) by comparing cage controls and ambient stromatolites with treatments that excluded all fish in experiment 2.

Results

The demonic intrusions of Hurlbert (1984) reduced the number of replicates surviving at the experimental harvest. Reasons for replicate loss ranged from escaped fish, wind damage, and anthropogenic intrusions (i.e. unintentional vandalism from curious kayakers). In experiment 1 (*H. minckleyi* manipulation), the numbers of replicates remaining were: 4 Molariform only, 8 Papilliform only, 9 Cage controls, and 9 No cichlids. In experiment 2 (pupfish manipulation) the numbers of replicates remaining were: 5 Pupfish only, 8 Cage controls, and 7 No fish.

Removal of invertebrates with CO_2 resulted in the collection of 30 taxa from all of the treatments (Appendix 1). We ran analyses on only the dominant invertebrate orders: Amphipoda, Diptera, Ephemeroptera, Gastropoda, and Trichoptera. Other taxa, such as the odonates and trombidiformes were found in such low densities that they were not analyzed separately. Additionally, throughout the experimental period, cage control variables resembled ambient variables indicating that there were no artifacts due to enclosures.

Fish morphs/species differed in their effects on snails and algae, both in direction and magnitude (Table 1). Molariform morphs had the biggest effect on both snails and algae, exerting strong controls on snails (-0.82), which cascaded into a net positive effect on stromatolite biomass (0.58). Papilliform morphs, in contrast, released snails from predation – causing a positive effect on snails (0.79). Surprisingly, although papilliforms have a positive effect on snails compared to the no cichlid treatments, the stromatolite algae had a small positive effect (0.14), and not a negative effect. Pupfish, without molariforms or papilliforms, had a negative effect on both snails (-0.16) and stromatolite algae (-0.43). The fish assemblage as a whole had effects similar to the molariform treatments – a strong negative effect on snails (-0.98) and a positive effect on stromatolite algae (0.30).

In experiment 1, molariform treatments had significantly fewer snails than treatments excluding molariforms (no cichlids and papilliform only) (Fig. 1c, Table 2). Other invertebrates significantly affected by molariform presence (versus papilliform presence) were an increase in overall densities (Fig. 1a), Amphipoda (Fig. 1b), and Diptera (Fig. 2b). Molariform treatments had significantly higher algal biomass than treatments that excluded cichlids (Fig. 3, ANOVA $F_{4,35} = 4.14$, $p = 0.0075$).

Papilliform treatments had different effects compared to molariform treatments. Papilliform treatments had a significant increase in snails compared to other treatments (460% increase compared to cage controls) (Fig. 1c, Table 2). The only other significant effect on invertebrates was a significant increase in Ephemeroptera (Fig. 2c). Stromatolite algae, however, was substantially lower in papilliform treatments relative to

ambient stromatolites, cage controls and molariform enclosures indicating that papilliforms do not cause a trophic cascade (Fig. 3).

In experiment 2, pupfish enclosures had no significant effect on most invertebrate groups (Fig. 4, Table 3), compared to other treatments, only significantly decreasing Trichoptera and Diptera densities, which likely had little effect on algal biomass (Fig. 5a,b). Average snail densities doubled in pupfish enclosures compared to cage controls and ambient stromatolites (Fig. 4c) and was marginally significant (ANOVA $F_{3,26} = 2.72$, $p = 0.065$). Pupfish enclosures significantly reduced stromatolite algal biomass by approximately half (Fig. 6; ANOVA $F_{3,26} = 4.28$, $p = 0.0139$). The same general patterns were seen in the exclusion of all fish – an increase in snails but no other strong effects on invertebrates. There was a non-significant decrease in algal biomass (Fig. 6).

Across all treatments, mean algal biomass significantly decreased as a function of mean snail density (slope significantly < 0, $t_7 = -2.44$, $p = 0.049$, Fig. 7). Overall, only stromatolites in the molariform treatments mimicked ambient stromatolites, suggesting molariform morph persistence is essential in maintaining stromatolites.

Discussion

The molariform morph of *H. minckleyi* is a keystone predator, essential for structuring stromatolites and associated invertebrate assemblages in Cuatro Ciénegas. Although all three fish had distinct and significant effects on stromatolites and invertebrates the strongest interaction chain extends from molariform fish through snails to stromatolites. We know of no other studies, where a subpopulation of a species plays a keystone role.

Comparisons of our cascade strength values to a meta-analysis looking at cascade strengths across a variety of ecosystems (e.g. lentic benthos, lentic plankton, terrestrial,

stream benthos, etc) (Shurin et al. 2002) indicates that our 2 comparisons that resulted in trophic cascades (Molariform enclosures vs. no cichlids, and cage controls vs. no fish) had similar effect sizes to other published experiments, although the effect size was closer to observed effect sizes in marine plankton and terrestrial ecosystems than stream benthic ecosystems.

Most notable is the contrasting effect of the two morphs of *H. minckleyi*. As predicted, the molariform morph decreased snails and increased algae through a trophic cascade, whereas the papilliform morph increased snails and slightly increases algae. In contrast to the molariform morph pupfish reduced both snails and algae which may be due to direct pupfish herbivory on stromatolites and competition with snails (see below). Although snails almost doubled increased when pupfish were excluded they were statistically non-significant, and did not match the 5 fold increase caused by molariform exclusion (with papilliforms). The 35% decrease in chlorophyll *a* in the presence of pupfish relative to treatments without fish is probably attributable to both direct grazing by pupfish and increased grazing by snails.

Snails were more important than other, more numerically dominant invertebrates in controlling stromatolites. For example, although amphipods almost quadrupled in treatments which excluded papilliforms (presumably from a lack of papilliform predation), there was no observable effect on primary production. The importance of gastropod grazers in other stromatolite forming systems has also been observed. In particular, Kinsman and Park (1976) noted that stromatolites in the Trucial Coast, Saudi Arabia only formed in areas where gastropods were absent due to high salinities – in

other nearby habitats, where salinities are lower, stromatolites were prevented due to gastropod grazing.

Are there threshold snail densities beyond which stromatolites cannot persist? Our results show clear reductions in stromatolite productivity under increased grazing pressure by snails and suggest that over time stromatolites in Cuatro Ciénegas could go extinct if molariform densities decreased and snails increased. Algal biomass dropped roughly 39% in treatments with the removal of molariforms but was never below 97 mg m^{-2}, a value that may still constitute actively forming stromatolites. Previous work however, comparing calcification rates of Cuatro Ciénegas stromatolite algae versus snail consumption ("bioerosion") found that calcification rates were only about 24% higher than bioerosive rates of snail consumption at ambient snail densities (Garcia-Pichel et al. 2004). Assuming that biomass estimates are directly related to calcification rates, the 39% decrease that we observed would likely be below the threshold calcification rate needed for stromatolites to generate calcification rates higher than snail consumption. Factor alongside this our observed increase in snail densities (as high as 460% increase – which represents a huge increase in the bioerosive capacity), and continued stromatolite formation is likely impossible.

Our results contrast experimental work by Elser et al. (2005) who studied the effects of phosphorous enrichment and elevated snail densities on Cuatro Ciénegas stromatolites. In a 7-week factorial experiment on stromatolites isolated in buckets, they added phosphorous while manipulating snail densities from 9 per stromatolite to 12 per stromatolite and found that phosphorus stimulated algal growth whereas the addition of 3 snails had no effect. Much of the discrepancy between their study and ours is likely due

to differences in snail densities. Their estimate of 9 snails per stromatolite (287 snails m^{-2}) is far below our estimate of 448 snails m^{-2} in ambient and cage control stromatolites. Hence, their treatment with "high" snail densities of 12 per stromatolite (or 382 snails m^{-2}) was still lower than our observed ambient densities. Fish removal increased snails to roughly 1200 snails m^{-2} showing the potential for snail densities to increase by over 300% if relieved from predator pressure. Garcia-Pichel et al. (2004) showed that it would take only a 33% increase in snail bioerosion to match stromatolite calcification, suggesting that the 33% increase would result in snail grazing matching accretion rates, but would not necessarily result in snail bioerosion surpassing stromatolite formation. Additionally, they used only one species of snail, *M. quadripalium*, and excluded the other commonly occurring snail, *N. minckleyi*, which sometimes outnumbers *M. quadripalium* on stromatolites. Finally, given the slow growth of stromatolites (~1-2 mm $year^{-1}$), 7 wks may not be long enough to discern potential long-term effects of snails on stromatolites. We conducted a similar fish exclosure experiment to the one described here that was only run for 12 weeks. This shorter experiment showed significant increases in snails but no effect on stromatolite biomass (Dinger et al 2006) indicating the importance of longer studies for indirect interactions to be manifested. Viewing these studies together, we concur with Elser et al.(2006), that under ambient snail densities, which are maintained at “low” levels by molariform fish, stromatolites are primarily phosphorus limited. However, if densities of this threatened fish decline, the balance could easily shift to strong grazer limitation.

Paleobiology implications

The Precambrian past differs in many ways from the modern environment of Cuatro Ciénegas (e.g. presence of higher trophic levels, different algal taxa involved in stromatolite formation), making many direct comparisons difficult. But one paleobiological observation that has, and can be made using Cuatro Ciénegas as a Precambrian analogue is that invertebrates and stromatolites co-occur. This has been used to suggest that invertebrate radiation and evolution were not the cause of declining diversity and distribution of stromatolites during the Cambrian transition (Farmer 1992). Here we show that stromatolites, invertebrates and invertebrate predators co-exist primarily because predators maintain grazer densities at low enough levels that the snails are released by grazing pressure. Without their vertebrate predators and the resulting trophic interactions, invertebrate grazers can reduce stromatolites, as is consistent with the invertebrate interference hypothesis for stromatolite decline.

Conservation biology implications

These results show that trophic interactions are essential for maintaining stromatolite growth, pointing to the importance of managing entire food webs rather than single species. In Cuatro Ciénegas, even if managers focused on what may be an appropriate species, *H. minckleyi,* efforts must be made to ensure that *both morphs* persist in the ecosystem. Persistence of both morphs depend on ensuring ample snail and soft-bodied invertebrate population, which in turn will maintain stromatolite growth. In other words, survival of stromatolites require that all levels of the food web are protected. However, in Cuatro Ciénegas, the morphs of *H. minckleyi* are threatened by non-native species (Tilapia – *Oreochromis sp.* and African Jeweled cichlids – *Hemichromis guttatus*)

and water extraction, both within the basin and by groundwater pumping outside the basin. Furthermore, the "keystone" morphotype, molariforms are already the less frequent morph found within the basin (Swanson et al. 2003). Monitoring and management plans must include studying the ratios of morphs, snail populations, and stromatolite growth to ensure that this unique ecosystem is preserved.

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Table 1. Cascade strength results as measured as ln ratios. Values represent the ln ratio of average snails (individuals m^{-2}) or Chl *a* (mg m^{-2}) between treatments. Positive values indicate positive trophic cascades (promoting growth), and negative values represent negative trophic cascades (preventing growth).

	ln (N_{p+}/N_{p-})	
Comparison	Snails	Chl *a*
Molariform/no cichlids	-0.82	0.58
Papilliform/no cichlids	0.79	0.14
Pupfish/no pupfish	-0.16	-0.43
All fish/no fish	-0.98	0.30

Table 2. Summary of invertebrate means and ANOVA results of dominant orders in *Herichthys minckleyi* experiment. Numbers are average number of individuals m^{-2}. Degrees of freedom = 4,35 for all ANOVAs.

	Treatment Means (±SE)						
Taxa group	Ambient	Cage Control	Molariform Only	Papilliform Only	No Cichlids	*F* ratio	*p* value
All Invertebrates	2436 (505)	2462 (314)	7820 (3657)	5619 (622)	3092 (583)	4.8152	0.0034
Amphipoda	1416 (425)	1557 (554)	6590 (3135)	3154 (536)	2027 (529)	4.5257	0.0047
Diptera	373 (30)	379 (41)	800 (297)	428 (82)	157 (45)	5.7254	0.0012
Ephemeroptera	17 (6)	19 (8)	20 (20)	65 (21)	19 (7)	2.7722	0.0422
Gastropoda	483 (74)	380 (110)	352 (226)	1775 (284)	803 (100)	14.433	<0.0001
Trichoptera	75 (12)	85 (30)	47 (18)	89 (20)	8 (4)	3.3454	0.0202

Table 3. Summary of invertebrate means and ANOVA results of dominant orders in *Cyprinodon bifaciatus* experiment. Numbers are average number of individuals m^{-2}. Degrees of freedom = 3,26 for all ANOVAs.

	Treatment Means (±SE)					
Taxa group	Ambient	Cage Control	Pupfish Only	No Fish	*F* ratio	*p* value
All Invertebrates	2436 (505)	3510 (872)	2505 (1098)	2968 (384)	0.5322	0.664
Amphipoda	1416 (425)	2342 (718)	1225 (522)	1150 (180)	1.14	0.351
Diptera	373 (30)	511 (121)	116 (48)	553 (103)	4.16	0.016
Ephemeroptera	17 (6)	52 (19)	3 (3)	14 (5)	3.5198	0.029
Gastropoda	483 (74)	497 (116)	1026 (547)	1201 (239)	2.7253	0.065
Trichoptera	75 (12)	51 (19)	10 (10)	15 (5)	5.3855	0.005

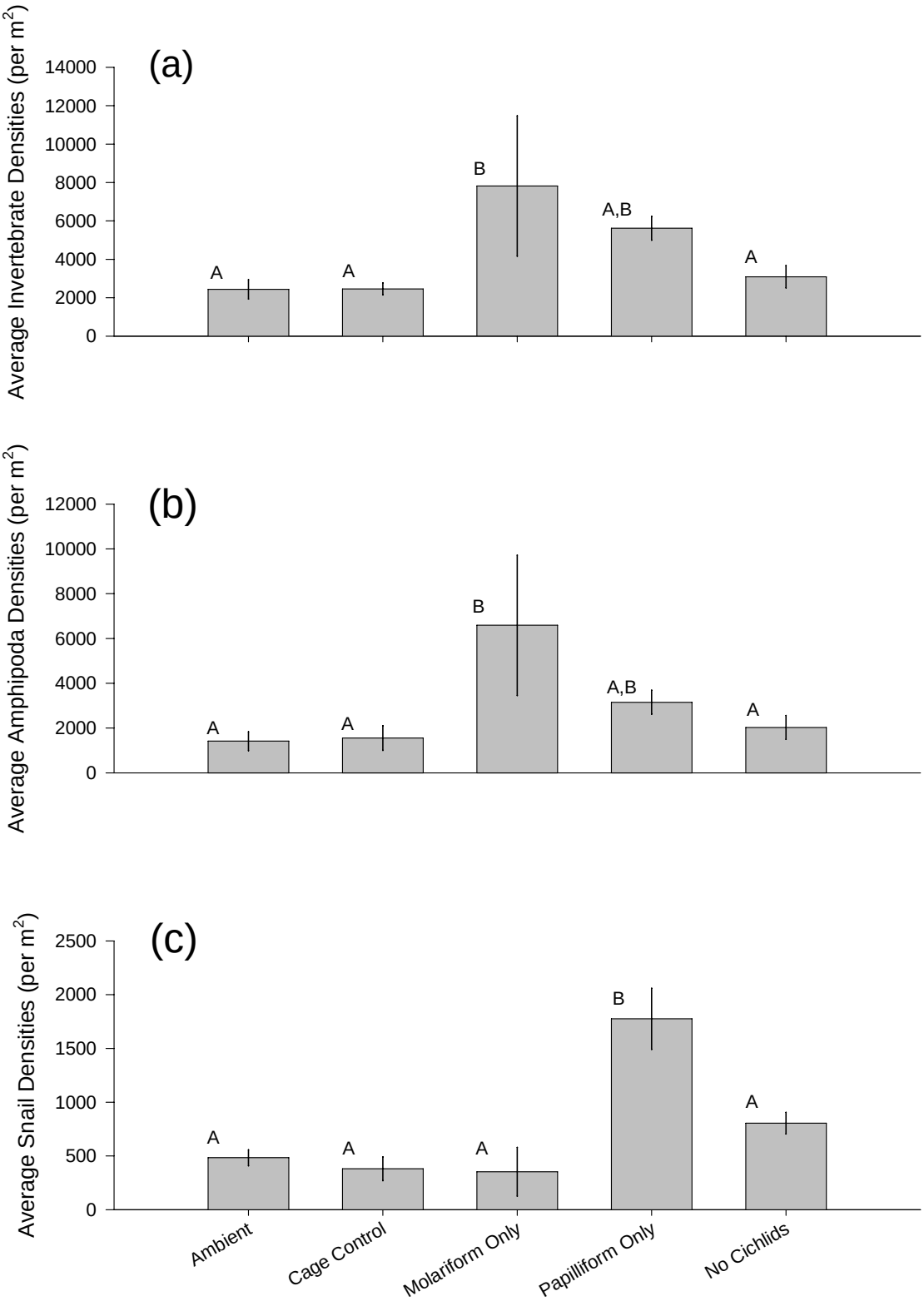

Figure 1. Average invertebrate response to *H. minckleyi* morph manipulation (± 1 SE) for (a) Total invertebrates, (b) Amphipods and (c) Gastropods. Different letters indicate significant differences (*Post – Hoc* Tukey HSD test). Overall means and ANOVA results are presented in Table 2.

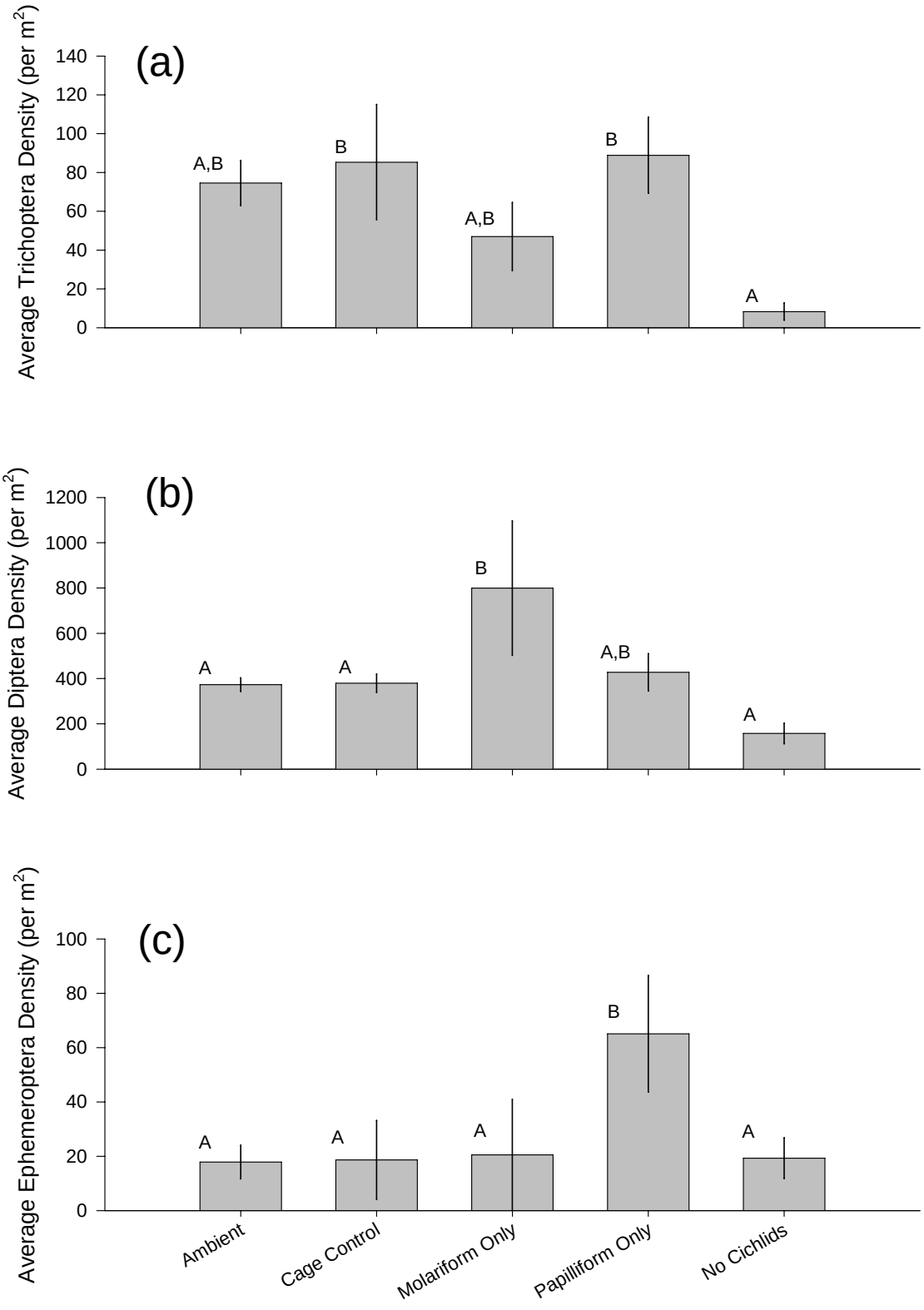

Figure 2. Average invertebrate response to *H. minckleyi* morph manipulation (± 1 SE) for (a) Trichoptera, (b) Diptera and (c) Ephemeroptera. Different letters indicate significant differences (*post-hoc* Tukey HSD test). Overall means and ANOVA results are presented in Table 2.

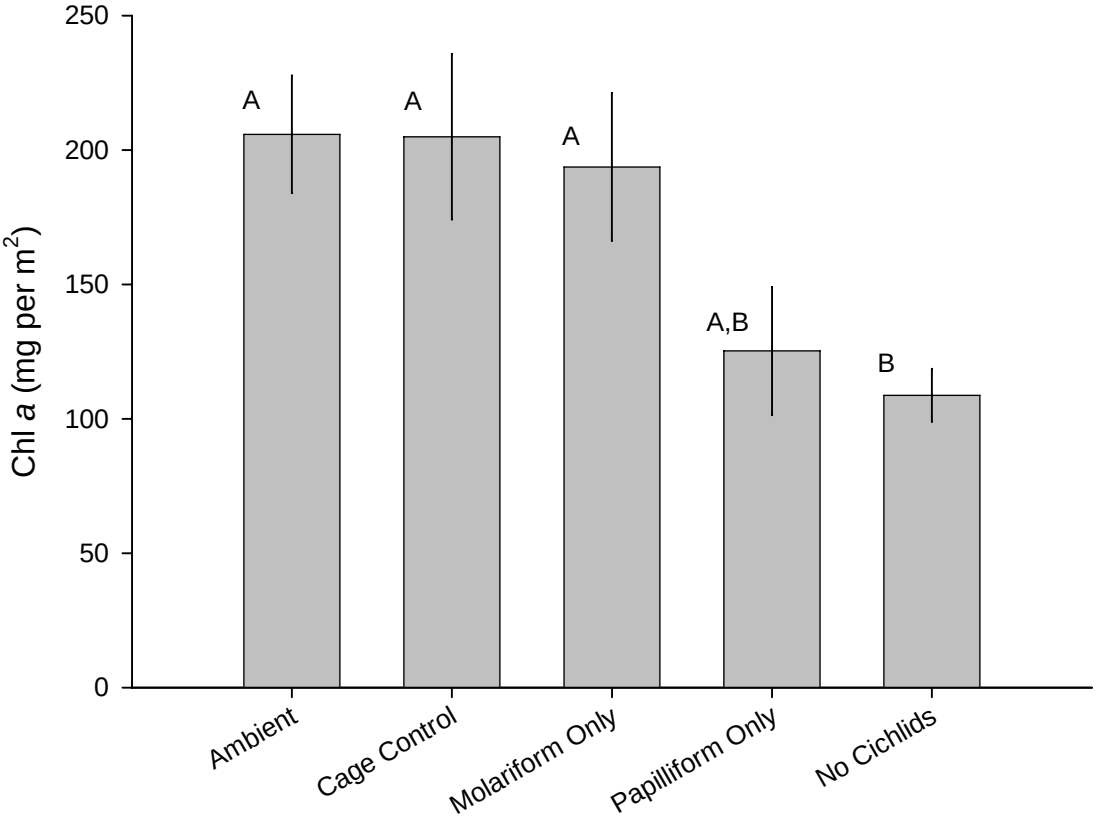

Figure 3. Average stromatolite algal biomass response to *H. minckleyi* morph manipulation (± SE). Different letters indicate significant differences (*post-hoc* Tukey HSD test).

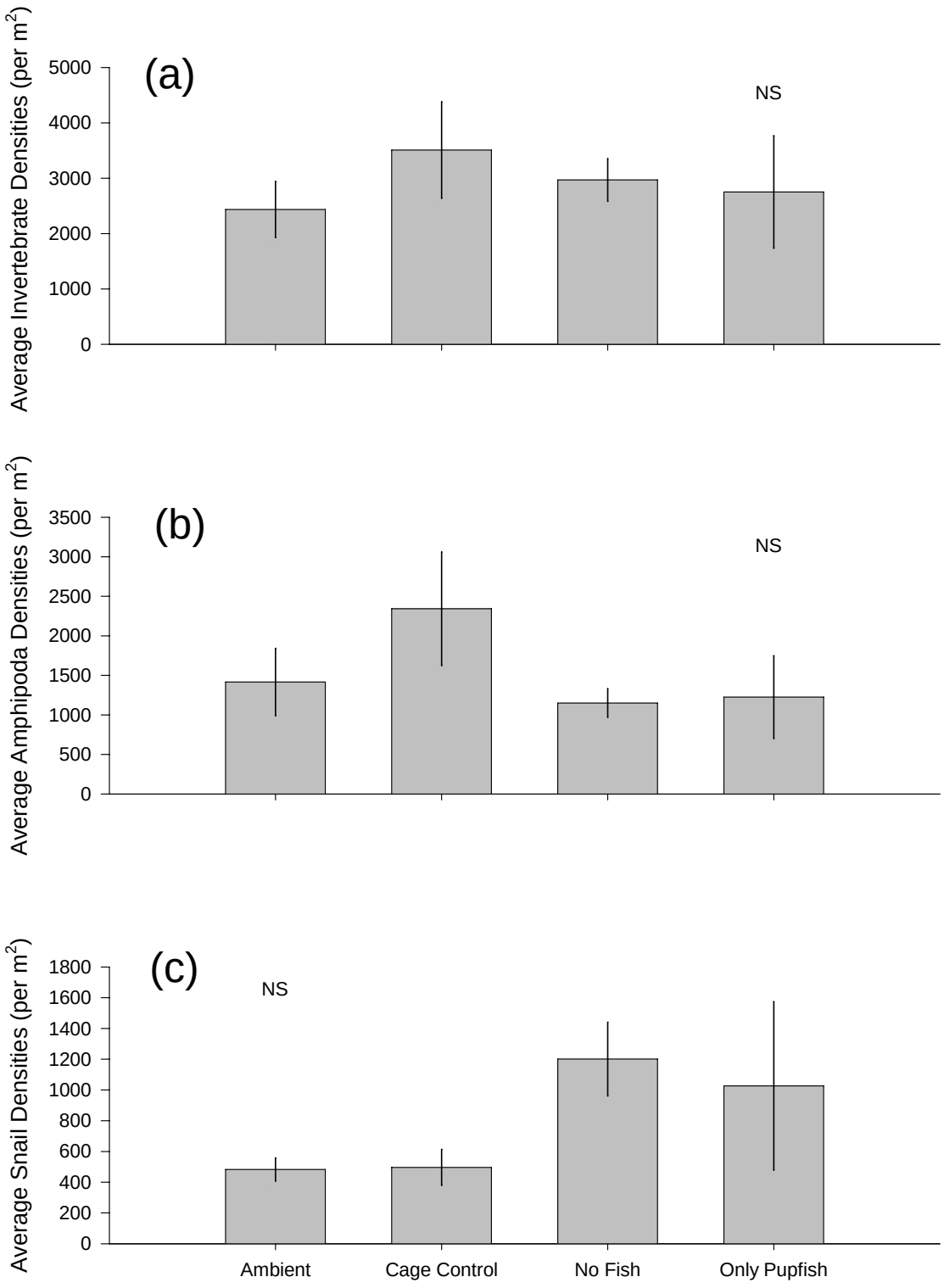

Figure 4. Average invertebrate response to *C. bifasciatus* manipulation (± 1 SE) for (a) Total invertebrates, (b) Amphipods and (c) Gastropods. Different letters indicate significant differences (*post-hoc* Tukey HSD test). Overall means and ANOVA results are presented in Table 3.

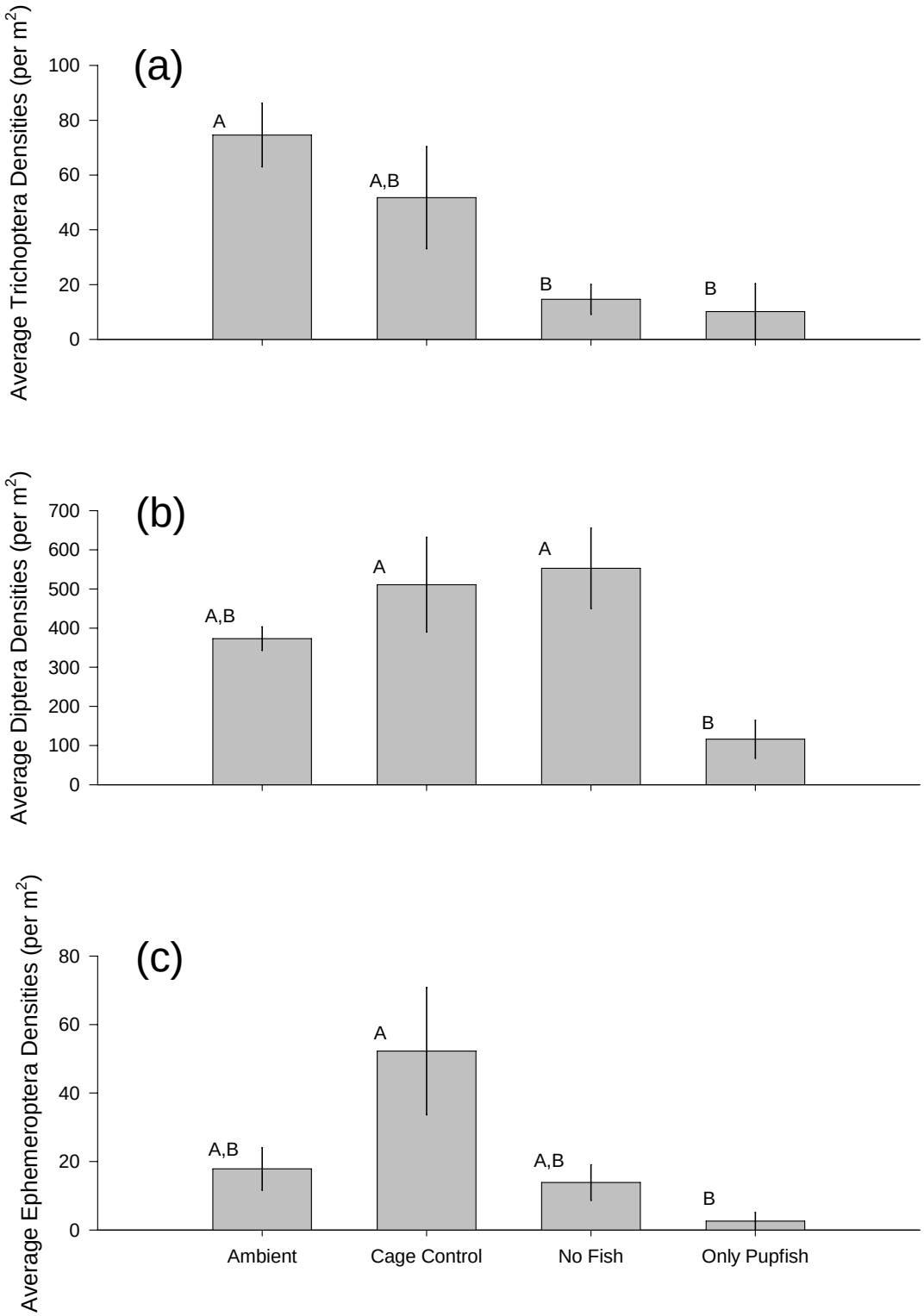

Figure 5. Average invertebrate response to *C. bifasciatus* manipulation (± 1 SE) for (a) Trichoptera, (b) Diptera and (c) Ephemeroptera. Different letters indicate significant differences (*post-hoc* Tukey HSD test). Overall means and ANOVA results are presented in Table 3.

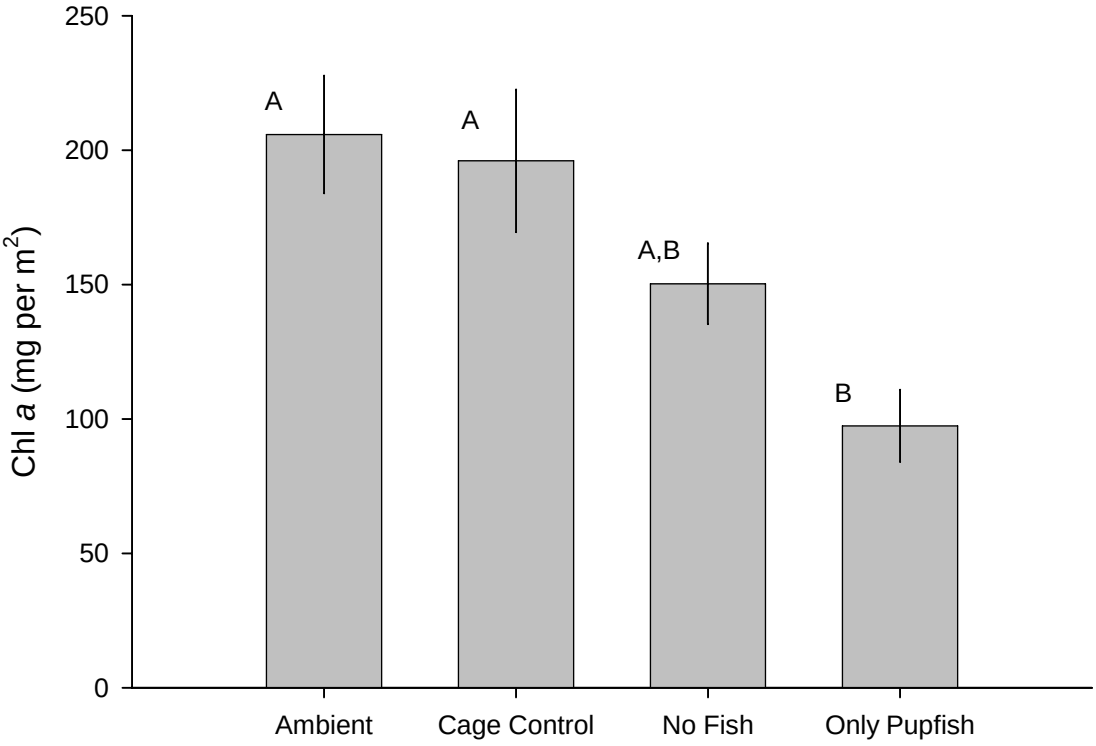

Figure 6. Average stromatolite algal biomass response to *C. bifasciatus* manipulation (± SE). Different letters indicate significant differences (*post-hoc* Tukey HSD test).

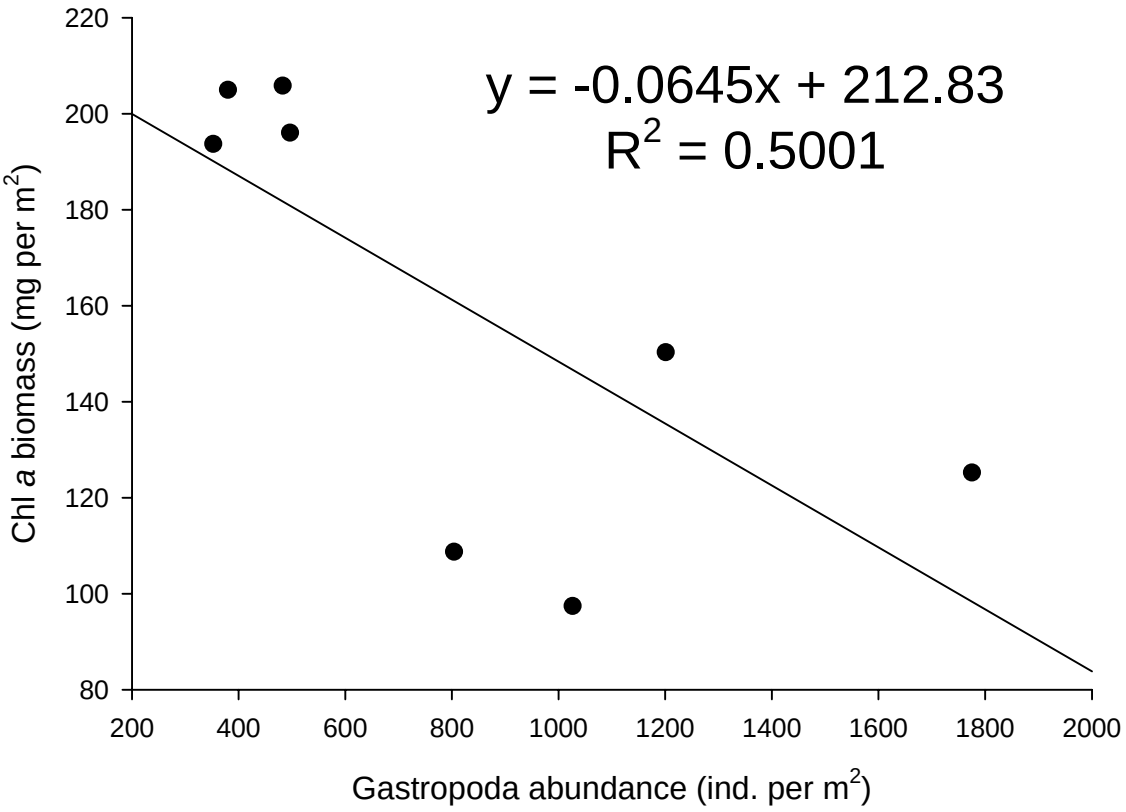

Figure 7. Stromatolite biomass (as Chl *a*) for all treatments from both experiments in relation to snail density (Gastropoda).

Appendix 1. Invertebrates collected from experimental stromatolites during harvest time.

Order Family Lowest taxonomic level	Order Family Lowest taxonomic level
Amphipoda	Gastropoda
Hyalellidae	Physidae
Hyalella sp.	*Physa* sp.
Annelida	Hydrobiidae
Unidentified oligochaetes	*Durangonella* sp.
Coleoptera	*Mexipyrgus carranzae*
Elmidae	*Mexithauma quadripalium*
Dryopidae	*Nymphophilus minckleyi*
Helichus sp.	Lepidoptera
Hydrophilidae	Pyralidae
Hydraenidae	Odonata
Octhebius	Coenagrionidae
Decapoda	*Argia* sp.
Palaemonidae	*Enallagma* sp.
Palaemonetes suttkusi	Libellulidae
Diptera	*Macromia* sp.
Ceratopogonidae	Ostracoda
Bezzia sp	Unidentified
Culicoides sp.	Trichoptera
Chironomidae	Hydroptilidae
Various spp.	*Oxytheira* sp.
Tipulidae	Leptoceridae
Tipula sp.	*Nectopsyche* sp.
Ephemeroptera	Polycentropidae
Baetidae	*Cernotina* sp.
Callibaetis sp.	Trombidiformes
Caenidae	Unidentified water mites
Caenis sp.	Turbellaria
Leptohyphidae	Unidentified flatworms
Tricorythodes sp.	
Leptophlebiidae	
Traverella sp.	

Antimycin A affects macroinvertebrates: mortality and recovery

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Abstract

Restoring native fish to freshwater habitats often requires removal of exotic fish using chemicals such as Antimycin A . Despite widespread use, there are limited field studies quantifying the effects of Antimycin A on aquatic macroinvertebrates. Laboratory bioassays indicate that Antimycin A is less toxic to invertebrates than other chemicals such as rotenone, particularly when it is used at low concentrations. These studies do not assess how diverse invertebrate assemblages respond to chemical treatment under real world applications where complex habitats require that relatively high concentrations need to be used. We studied the immediate and lingering 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 54 μg/L and 100 μg/L) on macroinvertebrate drift, densities and species composition. We used the Hilsenhoff Biotic Index, a measure of invertebrate pollutant tolerance, to study changes in species composition. At the highest dose (100 μg/L) Antimycin A increased drift five fold and immediately decreased invertebrate standing stocks in pools and riffles. Densities rebounded in riffles within five months but remained depressed in pools. At lower concentrations macroinvertebrate mortality, measured as increased drift, was 24-fold higher than pretreatment levels. At this lower concentration, however, macroinvertebrate densities in the benthos were not reduced. Under both concentrations, there were shifts in species composition towards more tolerant species. Although Antimycin A effects were mostly short-term, several species were extirpated. We caution managers contemplating the use of Antimycin A in fish renovations to consider the risks to macroinvertebrates. We suggest bioassays at

anticipated treatment levels to predict the effects upon macroinvertebrates, especially sensitive species. Where there are sensitive species, steps should be taken to reduce effects. Additionally, timing Antimycin A treatments with natural disturbances may help mitigate treatment effects.

Introduction

Exotic species are implicated as one of the primary threats to freshwater biodiversity worldwide (Allan and Flecker 1993, Richter et al. 1997). Exotic fish have displaced native fish throughout the Southwestern United States 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 exotic species is often magnified by habitat degradation, leading managers to consider removing non-natives in conjunction with habitat improvements (Brasher 2003, Ormerod 2003). Eradicating non-native fish usually requires chemical treatment, although there have been some successes in lakes with intensive netting (Knapp and Matthews 1998) and in streams with intensive electrofishing (Kulp and Moore 2000). The two main piscicides approved for fish kills are Rotenone and Antimycin A, which both inhibit cellular metabolism of exposed organisms. Rotenone has been used since the 1930's, but severe impacts to non-target organisms, plus negative public perception of Rotenone has led to many fisheries managers favoring Antimycin A, even though much less is known about its effects on other organisms (Finlayson et al. 2002).

Antimycin A, a fungal antibiotic, was discovered in the late 1940's, and it's potential for use as a piscicide was recognized in the early 1960's (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 viewed as a preferred fish toxicant to Rotenone due to three perceived advantages: 1) it is toxic to fish at low concentrations (usually μg/L, not mg/L needed for

Rotenone), 2) it degrades rapidly into non-toxic constituents (hours to days), and 3) it has low toxicity to non-target organisms, targeting fish more effectively (Finlayson 2002).

Most of the evidence supporting Antimycin A as a better piscicide is based on laboratory studies or small-scale field studies (Walker et al. 1964, Houf and Campell 1974, Snow 1974) with few field studies from large fish eradication projects (Gilderhus et al. 1969). Effects of Antimycin A on non-target organisms are still not fully 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 when there are effects, they are not long-term. These perceptions are based largely on government publications, which have not been peer reviewed and are often unavailable to the public. 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 concentrations exceeding 50 μg/L, on the invertebrate assemblage in Fossil Creek, Arizona.

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. We used invertebrate drift as an immediate measure of application effects and benthic samples from pools and riffles to measure immediate effects as well as longer-term impacts, four and five months following treatment. We predicted that Antimycin A would create high mortality

evidenced by higher drift rates during treatment and reduced standing stock immediately after treatment. We predicted that invertebrate densities would rebound within six months of treatment. We also hypothesized that the species composition of the community would shift to more tolerant invertebrates.

This is one of the most comprehensive studies of macroinvertebrate responses to Antimycin A. By drawing on a large pre-treatment 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 where managers are considering using piscicides.

Study site

Fossil Creek (Fig. 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 above sea level: 1304 m) create the majority of baseflow of 1.302 m^3/s, although scattered smaller springs along the length of the stream also contribute (Malusa et al. 2003). This spring water contains large concentrations of calcium bicarbonate and dissolved carbon dioxide (Table 1). During this 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.

Exotic 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 exotic fish removal took place during the fall of 2004 prior to decommissioning so

that it could be conducted under reduced flows. This study took place 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 there were no exotics. The predominant native fish above the dam are desert suckers (*Catostomus clarki*), speckled dace (*Rhinichthys osculus*) and headwater chub (*Gila nigra*). Below the dam, the headwater chub is replaced by the roundtail chub (*Gila robusta*), and the Sonoran sucker (*Catostomus insignis*) is also encountered. Exotic green sunfish (*Lepomis cyanellus)* extended from the confluence of the Verde River to approximately 1.6 km from the springs. Their uppermost limit marked the beginning of the treatment reaches. Exotic bass (*Micropterus dolomieui)* were abundant from the Verde River to the Irving Power Plant, where roughly 0.142 m^3/s water was returned to the creek increasing flow to 0.198 m^3/s. Closer to the confluence with the Verde River, two more exotic fish are present – flathead catfish (*Pylodictis olivaris*) and yellow bullhead (*Ameiurus natalis*).

Invertebrate Assemblages

Prior to restoration, Fossil Creek supported a diverse assemblage of macroinvertebrates with over 135 taxa found in the basin (Marks et al. 2005). Two species of special concern are found within the Fossil Creek drainage – a microcaddisfly (Trichoptera: Hydroptilidae), Metrichia *nigritta*, that is throughout Fossil Creek and an endemic Hydrobiidae (Gastropoda) snail (*Pyrgulopsis simplex*) limited to the Fossil Springs and several smaller springs within the drainage.

Methods

Renovation schedule and procedures

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 exotic fish (approximately 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 where the Bureau of Reclamation constructed a fish barrier. Native fish were salvaged from both reaches prior to chemical treatment, transported by helicopter to a holding facility, and returned to the river after chemical treatment.

The first reach was treated with Antimycin A (Finitrol ®) on the 19th and 20th of October 2004. Antimycin A target dosage was 50 μg/L in the main channel, but the bottom of the reach had a target dosage of 100 μg/L (increased due to high iron in the water). Additional treatment of side channels was at 10 μg/L. Application was accomplished using bucket drip stations approximately 150 m apart, during a 4-hour exposure period following instructions recommended by the manufacturer. In addition to the drip stations, Antimycin A was applied in two other manners. 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 – with renovation crews 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 done using a drip station of Potassium Permanganate (3 mg/L) at the bottom of the treatment reach.

Treatment of reach 2 was conducted in a similar manner with some exceptions. Although the initial application plan called for a concentration of 100 µg/L, increased stream flow from recent precipitation diluted the actual application concentration. Managers were unable to secure additional Antimycin A and proceeded with the treatment plan using quantities calculated under normal base flow. The upper portion of the reach was treated on 9 November 2004 at application concentration of 35 µg/L. The lower section was treated on 10 November 2004 when water levels were lower resulting in an application of 54 µg/L. Backpack sprayers and Antimycin A laden sand were applied as described above. Detoxification used sodium permanganate instead of potassium permanganate, at 3 mg/L.

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 had been sampled six times in the two years prior to restoration. In treatment reach 1 the impact site, which was directly above the Irving Power Plant, experienced target applications of Antimycin A of 100 µg/L. In treatment reach 2 the impact site was just downstream of the middle of the 2nd treatment 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 meters 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.

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 a 250 μm mesh, following standard protocols (Hauer and Resh 1996). Samples were taken at haphazard locations within the study reach. Invertebrates and substrate (cobble, gravel, particulate matter) collected in the Surber sampler were transferred to a 5-gal 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 a haphazard location. A trowel was used to retain the sample while transferring it to a 5-gal 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 ten replicates were taken in the periods leading up to and following the Antimycin A treatment (Table 2). In the laboratory, invertebrates were sorted with a magnifying glass and identified to the lowest practical taxonomic level (usually genus, except for Chironomidae which was left at family) and enumerated.

Drift Samples

Drifting invertebrates were collected following standard protocols (Smock 1996) with two different net designs – one large design by a commercially available manufacturer and one small homemade design. The manufactured design was purchased at Aquatic Ecosystems and measures 30 cm by 30 cm, with either a 250 μm or a 500 μm mesh net. The homemade designs were 14 cm by 14 cm with 500 μm mesh net. 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 minutes, 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 1mm mesh sieve to standardize for different mesh sizes of the nets. Invertebrates were sorted and counted and reported as the # individuals per 100 m^3 of water (Smock 1996).

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. The Hilsenhoff Biotic Index is a weighted average of tolerance values for each sample (Hilsenhoff 1987, 1988) and is calculated as:

$$HBI = \Sigma n_i t_i / N$$

where n_i = number of individuals counted for species I, t_i = tolerance value for species i, and N is the total number of individuals in a sample. Tolerance values range from 0 to 10, with 0 being intolerant of stress, and 10 being very tolerant of stress. Tolerance

values for invertebrates in our samples were taken from regional values developed for the US Environmental Protection Agency (Barbour et al. 1999). Because no values have been developed for the Southwest, we used the values developed for the Northwest. When no value existed for the Northwest, we used Midwest values. 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 following treatment indicate a shift in community composition to more tolerant species.

Statistical analyses

Significance of short-term HBI and density changes for both benthos and drift were analyzed with ANOVA analyses using a standard Before-After-Control-Impact (BACI) model (Green 1979). This method uses both time (before and after) and site (control and 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 in that it was a Before-*During*/Control-Impact design. All density data used in the analyses were log (x +1) transformed to normalize variances.

We used a Before-After-Control-Impact 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 uses time (Before-After) as the sole factor. Because our drift samples were limited to before and during the treatment, this analysis was not applied to the drift. All ANOVAs were analyzed 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 Non-Metric Multidimensional

Scaling ordination (NMDS) routine in PC-ORD (version 4.02). These ordinations provide a graphical representation of community differences using the Sorenson (Bray-Curtis) distance measures. In the case of drift, we relativized the data to sample maximum to adjust for potentially large density differences between pretreatment and during treatment samples. To assist in the interpretation of these ordinations, we used the joint plot function of PC-ORD using the following secondary variables: Total Species Richness (SR), Eveness, Shannon's H, Simpson's D, Coleopteran SR, Dipteran SR, Ephemeropteran SR, Trichopteran SR, Other (miscellaneous taxa) SR, and the Hilsenhoff Biotic Index. 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 an 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 if there were any species that were only present in the benthos prior to treatment, and had failed to either persist or recover following treatment. In interpreting the results, we considered only species that were present prior to treatment, but were absent in 100% of the post-treatment samples. Significance of the indicator species analysis is 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). Drifting invertebrates significantly increased from a pretreatment average of 83.5 ind/100 m^3 of water to 443.2 ind/100 m^3 of water during the treatment, a more than five fold increase (BACI ANOVA; $F_{1,55} = 4.075$, $p = 0.048$) (Fig. 2A). Observations of drifting invertebrates indicated that the majority were dead upon collection. The HBI values of the during treatment invertebrate assemblage were not significantly different from pretreatment values (BACI ANOVA; $F_{1,53} = 2.372$, $p = 0.129$).

Invertebrates were scarce in samples taken initially after treatment. Densities of riffle invertebrates significantly dropped from pretreatment values of 2,802 ind/m^2 to 300 ind/m^2 (BACI ANOVA; $F_{1,39} = 8.41$, $p = 0.007$)(Fig. 2B). Riffle HBI also increased from 4.98 to 6.98 (BACI ANOVA; $F_{1,39} = 18.062$, $p < 0.001$) indicating a shift to more tolerant species. The effects in riffles were mirrored in pools, with a drop in density from 3,162 ind/m^2 pretreatment to 610 ind/m^2 after treatment, a five fold drop in density (BACI ANOVA; $F_{1,39} = 4.624$, $p = 0.038$). There was no effect on the HBI in pools (BACI ANOVA; $F_{1,39} = 0.035$, $p = 0.585$) (Fig. 2C).

Macroinvertebrates mostly rebounded within five months after treatment. There were no detectable long-term effects either in density or HBI in riffles (Table 3). Five months after the treatment, riffle invertebrates had increased to 3,326 ind/m^2, a value slightly higher than the immediate pretreatment value (2,802 ind/m^2). HBI had similarly rebounded to values similar to pretreatment levels within two months. Pool invertebrates

had also increased from post treatment lows of 610 ind/m^2 to 1,410 ind/m^2 five months after treatment. Although densities had not yet 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 (Fig. 3). The ordinations shows clear grouping of samples taken shortly after treatment, compared to the pretreatment and samples taken five months following treatment, indicating that the assemblages changed following treatment and then recovered close to a pretreatment state. Furthermore, the joint plot explanatory variables with an r^2 higher than 0.4 were HBI tolerance values (Toler) = 0.59, ephemeroptera diversity (E div) = 0.42, and species richness (SR) = 0.48. In this configuration, higher tolerances are associated with the samples taken shortly after treatment, whereas the pretreatment and five months later samples are associated with increased species richness and mayfly diversity.

Treatment Site 2

Like Treatment Site 1, application of Antimycin A resulted in immediate, large increases of drifting invertebrates (BACI ANOVA; $F_{1,57} = 31.582$, $p < 0.001$) (Table 4, Fig. 4A). This increase was much larger than the increase in Treatment Site 1, being an almost 24 fold increase (23.2 ind/100 m^3 water prior to treatment, 556.3 ind/100 m^3 water during treatment). Visual inspection again indicated that the majority of invertebrates were dead upon collection. Although the HBI of drift was not significantly different (BACI ANOVA; $F_{1,53} = 3.85$, $p = 0.055$), there was a trend of lower HBI in the invertebrates drifting during the treatment (average of 4.76) compared to HBI values

drifting prior to treatment suggesting that less tolerant species were more strongly affected by chemical treatment (average of 5.48).

Unlike Treatment Site 1, there was no short term effect of Antimycin A application on densities either in riffles or pools (Riffle: BACI ANOVA; $F_{1,39} = 0.023$, $p = 0.881$, Pool: BACI ANOVA; $F_{1,39} = 0.037$, $p = 0.848$) (Table 4, Fig. 4B,C). Not surprisingly, there was no long term effect either (Riffle: BACIPS ANOVA; $F_{1,7} = 0.007$, $p = 0.937$; Pool: BACIPS ANOVA; $F_{1,7} = 2.841$, $p = 0.144$). There were however short 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 (Fig 5). The joint plot function shows that the pretreatment drift was associated with higher HBI tolerance values ($r^2 = 0.37$), and that drifting invertebrates during treatment had relatively lower HBI tolerance values indicating that less tolerant organisms as measured by HBI are more susceptible to Antimycin A.

Ordination of the riffle assemblages (Fig. 6) shows clear separation between pretreatment samples and samples taken shortly after treatment. In contrast to treatment 1 at this site, samples taken four months later still did not resemble the pretreatment samples indicating that the assemblage did not yet fully recover. Joint plot function shows that pretreatment assemblages were associated with high Ephemeroptera diversity (E div; $r^2 = 0.528$) and that samples four months after treatment were associated with high Diptera diversity (D div; $r^2 = 0.53$).

Control Site

Relative to Treatment Sites 1 and 2, there was little change in the drift, density, or community (as measured by HBI) during the treatment periods (Fig. 7). Control drift rates were similar to pretreatment drift rates during the 1st treatment (Treatment Site 1 average: 83.4 indiviuals/100 m^3 water; Control Site average: 73.7 indiviuals/100 m^3 water) as well as the 2nd treatment (Treatment Site 2 average: 23.3 individuals/100 m^3 water; Control Site average: 17.3 individuals/100 m^3 water). The decrease in drift for the period between 13 October 2004 and 8 November 2004 was likely 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 after over four or five months had still not reappeared, whereas 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 found to be significant using Monte Carlo randomization. Treatment site 2 had eight taxa extirpated, with three being significant. In treatment site 1, this represented a loss of 7% of the total diversity, and treatment site 2 lost 14% of the total diversity.

Discussion

Our results show that Antimycin A can detrimentally affect macroinvertebrates depending on the concentrations of Antimycin A used and the species composition of the macroinvertebrates. Initial mortality rates during treatment were high and dramatically reduced densities. Nevertheless, densities recovered after five months at one site and

remained only slightly depressed at a second site where higher concentrations were used. Some species were particularly vulnerable to chemical treatment, failing to recover after five 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 assemblage at site 2 as measured by the HBI index. Given additional time, we expect full recovery in the invertebrates in both sites, This study was limited in duration by the flow restoration which commenced in June 2005. We will continue to monitor invertebrates particularly to see if the species that were not found after treatment recover. We anticipate however major changes in density and species composition of invertebrates with increased base flow, challenging our ability to detect lingering effects of Antimycin A. Nevertheless this study remains one of the most comprehensive evaluations of Antimycin A on invertebrates.

It is difficult to predict *a priori* which species may be extirpated from the community. Some species were locally extirpated in treatment site 1, but persisted in treatment site 2, and vice versa. HBI tolerance values were also not useful in predicting species losses. The average HBI tolerance values for the species lost was 4.3 for treatment site 1, and 4.2 for treatment site 2, which are 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). Both studies reported recovery of common taxa within one to three years (Jacobi and Deagan 1977, Minckley and Mihalick 1981). Species responses were not reported in the first study, whereas Minckley and Mihalick (1981) reported that six

species were still absent after three years which they attribute to sampling errors or possibly flooding events. Our more in-depth analysis, however, revealed that the absences of some species in Fossil Creek are unlikely due to sampling error or natural disturbance but are due to the chemical treatment. In contrast to our results, other researchers report no effect of Antimycin A, probably because these studies were conducted at lower concentrations (10 µg/L Antimycin A) (Gilderhus et al. 1969, Morrison 1979, Minckley and Mihalick 1981). Some of these studies were limited to artificial pools, with few to no controls (Snow 1974, Houf and Campbell 1977).

Interestingly, some government studies that concluded that Antimycin A is safe for invertebrates actually documented mortality rates of 50% to 99% but nevertheless concluded that Antimycin A is "largely specific to fish and causes no harm to most of the other aquatic animals" (Gilderhus et al. 1969, page 20), and that there are "no grossly toxic effects" (Walker et al. 1964, page 14).

Recovery in Fossil Creek was likely 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. Second, the AZGFD department timed the project for the late fall when many Arizona streams experience heavy rain and flash floods. This is a process that desert aquatic invertebrates are adapted to – either by finding refuge in the hyporheic zone, or in different life stages (e.g. terrestrial adults). 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 foul, fall weather was not easy, by placing the

artificial disturbance in the background of natural disturbance, overall effects to the biota may have been minimized.

The use of Antimycin A to eradicate exotic fish increased in the 1990's as managers confronted the difficult issues of conserving native fish, a trend that will likely continue (Finlayson 2002). Higher concentrations will need to be used as managers attempt to eradicate exotic fish in larger more complex rivers such as Fossil Creek. In most cases environmental assessments are required challenging managers and other stakeholders to consider the positive and negative effects on the health of the entire ecosystem.

We maintain that there is a misconception that Antimycin A does not affect aquatic invertebrates. Our results 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 mandatory 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 the Environmental Assessment (USDA 2003) and were not included in the subsequent monitoring program, in part because of the commonly 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.

We offer four general lessons that emerged from this study that can be applied to 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 prior to treatment. Second, in projects where native fish are reintroduced into the river from other sites or captive populations, it may be prudent to wait at least six months, while macroinvertebrates recover, 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 while they determined the necessary Antimycin A concentrations. To do this we measured drift in small test reaches. We were able to advise that high mortalities of aquatic invertebrates would likely 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 so that there is minimal effect on aquatic invertebrates, but high enough so that 100% fish kill is ensured. When the amount of Antimycin A needed is high enough so that large kills of invertebrates are unavoidable, this may suggest the use of alternative piscicides, such as the more accessible and cost effective Rotenone. This suggestion is based on the premise that one of the main advantages of Antimycin A over Rotenone is lower mortalities on aquatic

invertebrates. Fourth, when possible, projects should be timed to coincide with natural disturbance regimes or when the majority of insects are in their terrestrial life stage.

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Table 1. Physical and chemical characteristics values for study sites. Values are means of samples taken seasonally from August 2002 to May 2003.

Parameter	Control Site	Treatment Site 1	Treatment Site 2
Temperature (°C)	19.4	17.9	17. 7
Dissolved O_2 (mg l^{-1})	7.13	7.59	7.65
PH	8.12	8.2	8.37
Conductance (μS cm^{-1})	612	574	521
NH_3 (mg l^{-1})	0.04	0.03	0.03
PO_4 (mg l^{-1})	0.04	0.07	0.03
Salinity (ppt)	0.34	0.29	0.26
NO_3-N (mg l^{-1})	1.32	0.06	0.12
Mg (mg l^{-1})	37.5	40.5	17.6
Ca (mg l^{-1})	78.4	50.9	40.16
Na (mg l^{-1})	11.0	14.4	11.6
K (mg l^{-1})	1.73	2.24	1.79
Cl (mg l^{-1})	7.34	9.09	7.71
SO_4 (mg l^{-1})	22.4	15.3	24.1
CO_2 (mg l^{-1})	32. 7	27.4	28.3
Alkalinity (mg l^{-1})	383	296	246
NTU	1.16	2.6	1.71

Table 2. Treatment and sampling schedule for Antimycin A treatment of Fossil Creek. Numbers in parentheses indicate the number of replicates taken in that period. Benthos sampling numbers are for each type of sample (Pool and Riffle). * indicates Drift control dates for Treatment Site 2. NA indicates not applicable.

			Benthos Dates		Drift Dates	
	Treatment Concentration	Treatment Date	Pre	Post	Pre	During
Control Site	0 ppb	NA	8/14/2002 (5) 12/4/2002 (5) 10/1/2003 (5) 5/5/2003 (5) 1/31/2004 (5) 10/13/2004 (10)	11/19/2004 (10) 12/14/2004 (10) 3/17/2005 (10)	10/13/2004 (10) 11/8/2004 (10)*	10/19/2004 (21) 11/10/2004 (16)*
Treatment Site 1	>100 ppb	10/19/04	8/15/2002 (5) 12/16/2002 (5) 5/5/2003 (5) 9/30/2003 (5) 1/30/2004 (5) 10/18/2004 (10)	11/4/2004 (10) 12/13/2004 (10) 3/16/2005 (10)	10/18/2004 (10)	10/19/2004 (18)
Treatment Site 2	>54 ppb	11/10/04	8/12/2002 (5) 12/16/2002 (5) 5/5/2003 (5) 9/30/2003 (5) 1/30/2004 (5) 11/5/2004 (10)	12/13/2004 (10) 3/16/2005 (10)	11/5/2004 (10)	11/10/2004 (24)

Table 3. Results of ANOVA tests at Treatment Site 1 on changes in total invertebrate densities and changes in HBI from Antimycin A treatment. For BACI tests (Before-After-Control-Impact) only the interaction (site X date) is given.

Sample	ANOVA Type	Response Variable	Degrees of Freedom	F - Value	*p* - Value
Drift	Immediate (BACI)	Density	1, 55	4.076	0.048
		HBI	1, 53	2.372	0.129
Riffles	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
Pools	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

Table 4. Results of ANOVA tests at Treatment Site 2 on changes in total invertebrate densities and changes in HBI from Antimycin A treatment. For BACI tests (Before-After/Control-Impact) only the interaction (site X date) is given.

Sample	ANOVA Type	Response Variable	Degrees of Freedom	F - Value	*P* - Value
Drift	Immediate (BACI)	Density	1,57	31.582	<0.001
		HBI	1,53	3.854	0.055
Riffles	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
Pools	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

Table 5. Results of indicator species analysis for control and treatment sites showing extirpation of invertebrates following Antimycin A treatment. All listed taxa failed to show in any post treatment sampling. Extirpations significant by Monte Carlo simulation are denoted with a *. Percent Affected is the proportion of missing taxa to total no. of taxa appearing before treatment. E = Ephemeroptera, D = Diptera, T = Trichoptera, O = Odanata, C = Coleoptera, H = Heteroptera, L = Lepidoptera

Control		Treatment Site 1		Treatment Site 2	
Extirpated Taxa	*p* - Value	Extirpated Taxa	*p* - Value	Extirpated Taxa	*p* - Value
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	*Hemerodromia* (D)	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%

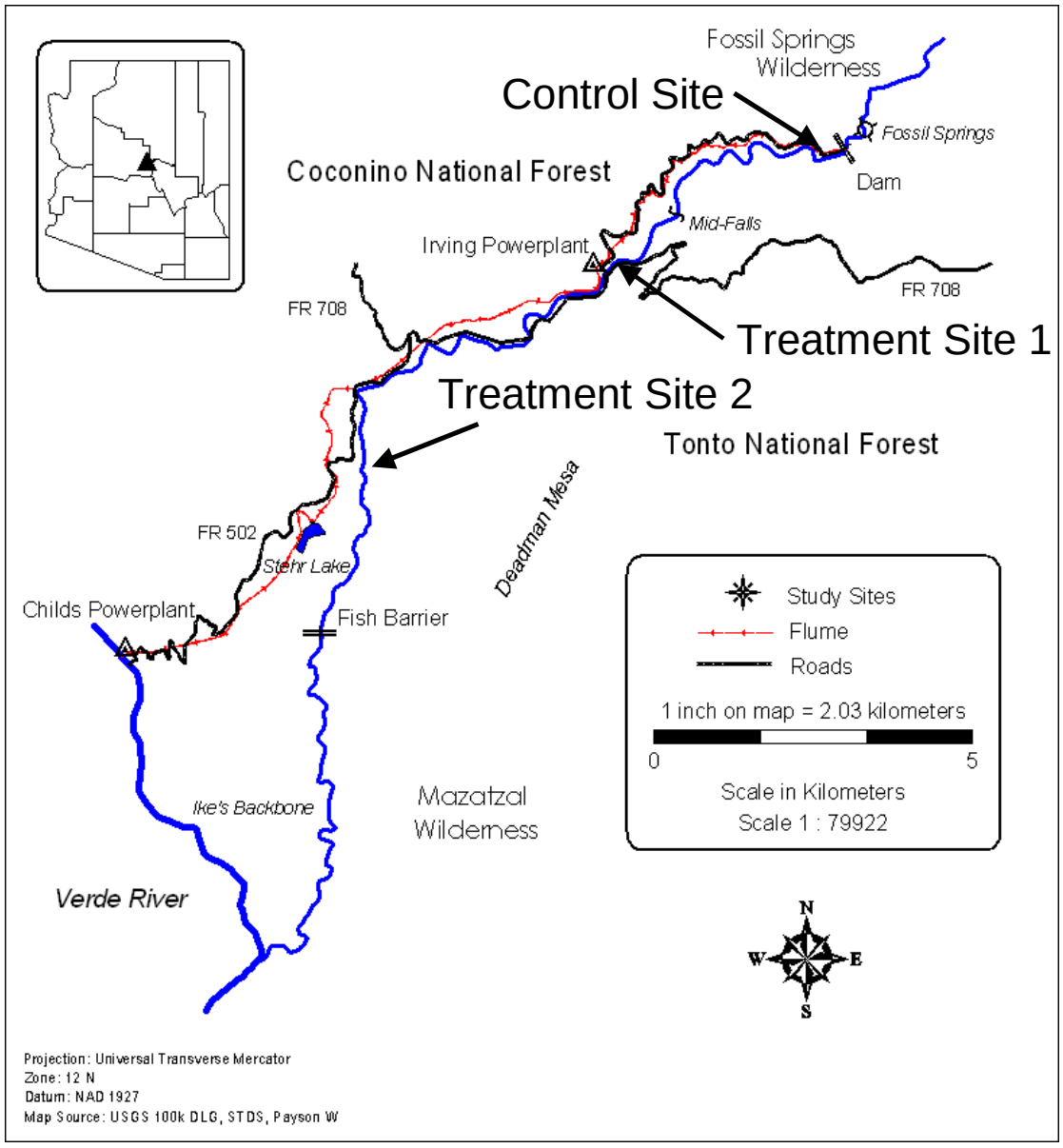

Figure 1. Map of Fossil Creek, Arizona showing study sites.

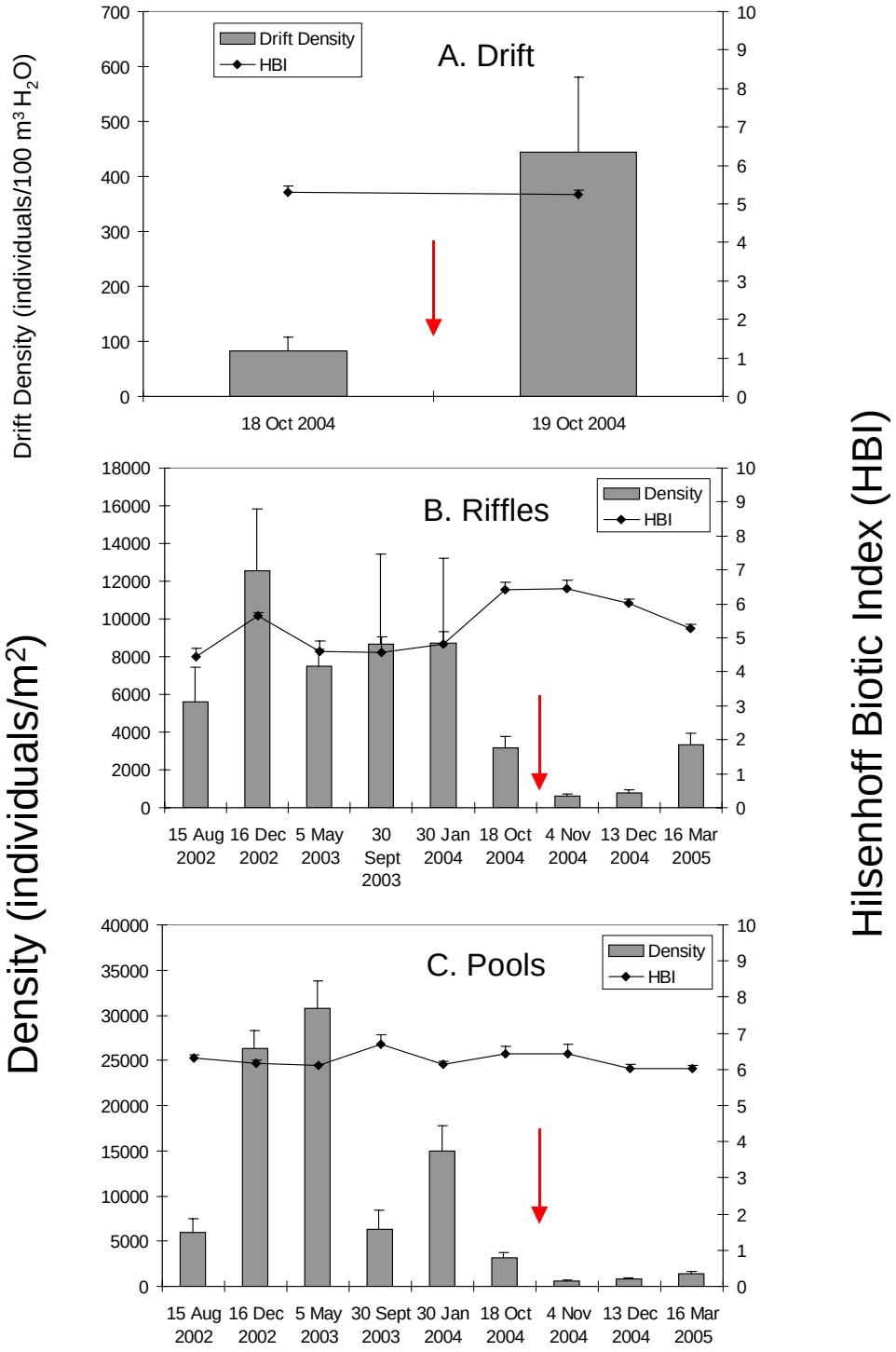

Figure 2. Mean drift, benthos densities and HBI in Treatment Site 1. A (top) is drift and HBI before and during treatment. B (middle) is densities and HBI in riffles before and after treatment. C (bottom) densities and HBI in pools before and after treatment. Treatment is indicated by red arrow. Bars = 1 SE.

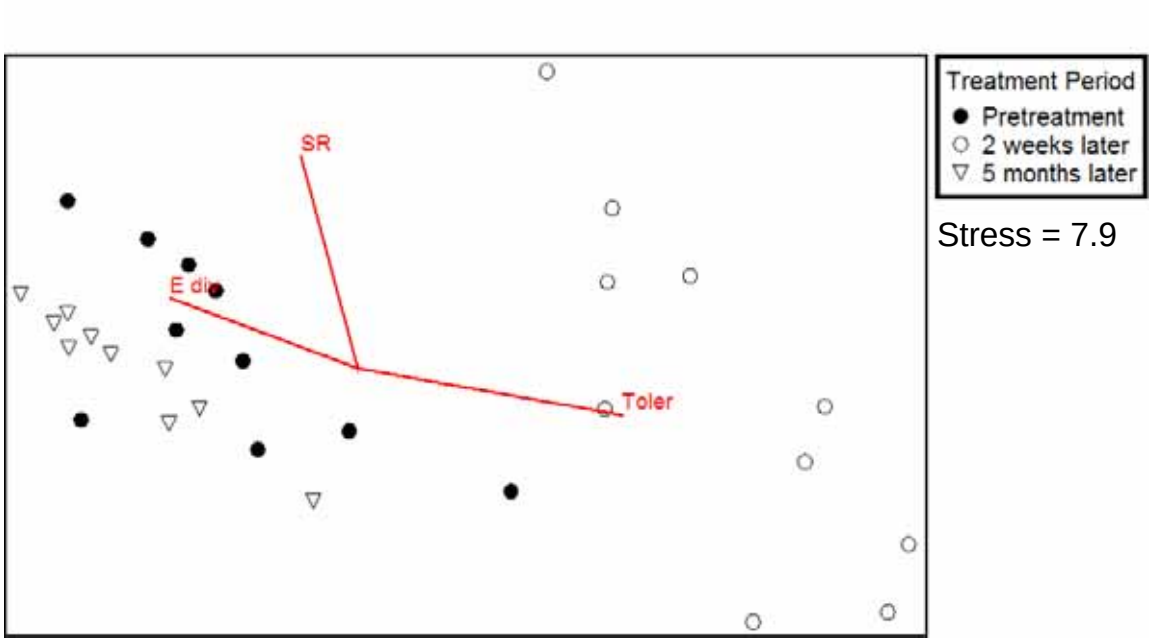

Figure 3. Non-Metric Multidimensional Scaling ordination of invertebrate riffle assemblages in Treatment Site 1 showing relationship of samples. Joint plots shown in red are variables that explained more that 40% variability ($r^2 > 0.4$) along axes 1 or 2. Toler = HBI tolerance values, SR = Species Richness, E div = Ephemeroptera diversity.

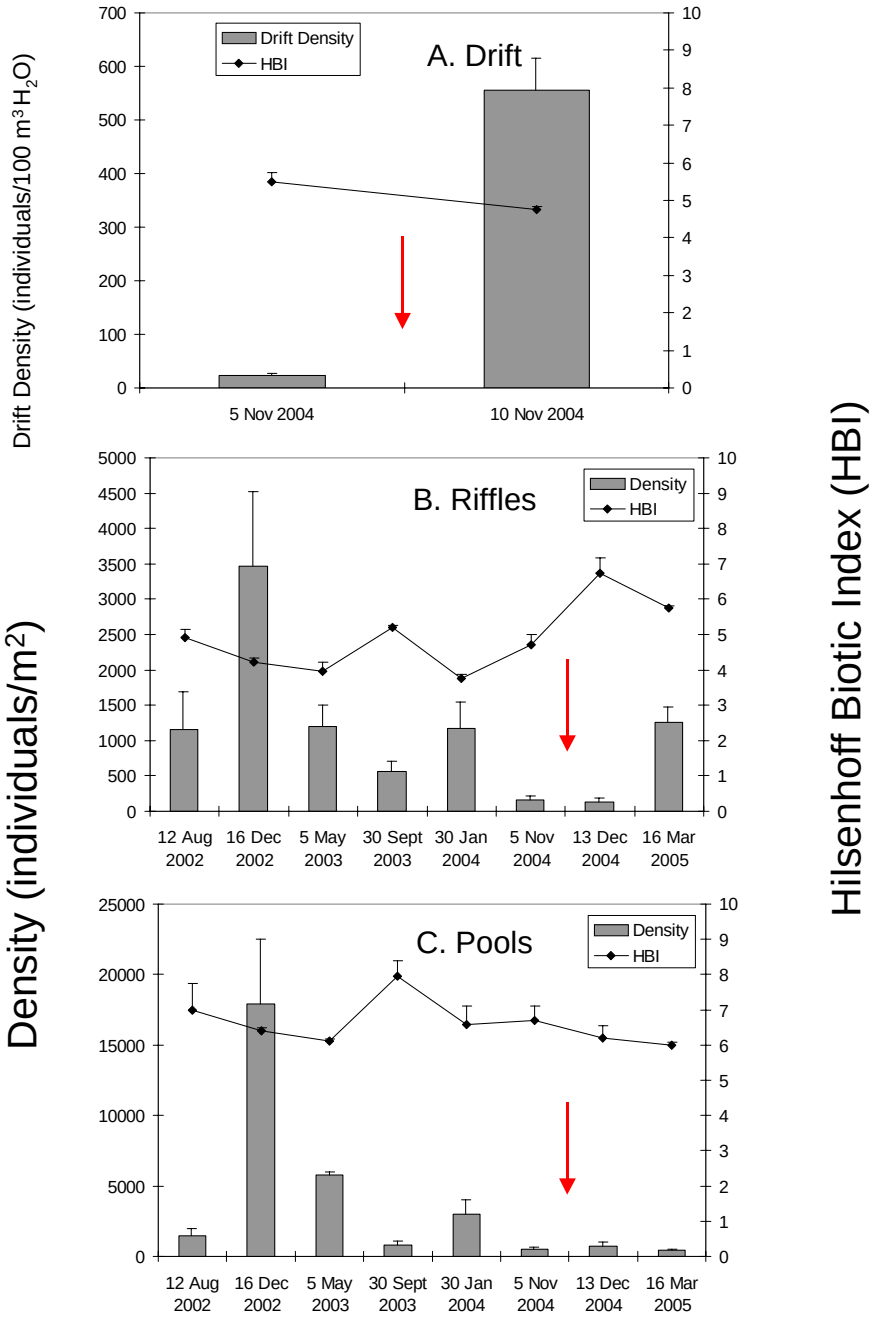

Figure 4. Mean drift, benthos densities and HBI in Treatment Site 2. A (top) is drift and HBI before and during treatment. B (middle) is densities and HBI in riffles before and after treatment. C (bottom) densities and HBI in pools before and after treatment. Treatment is indicated by red arrow. Bars = 1 SE.

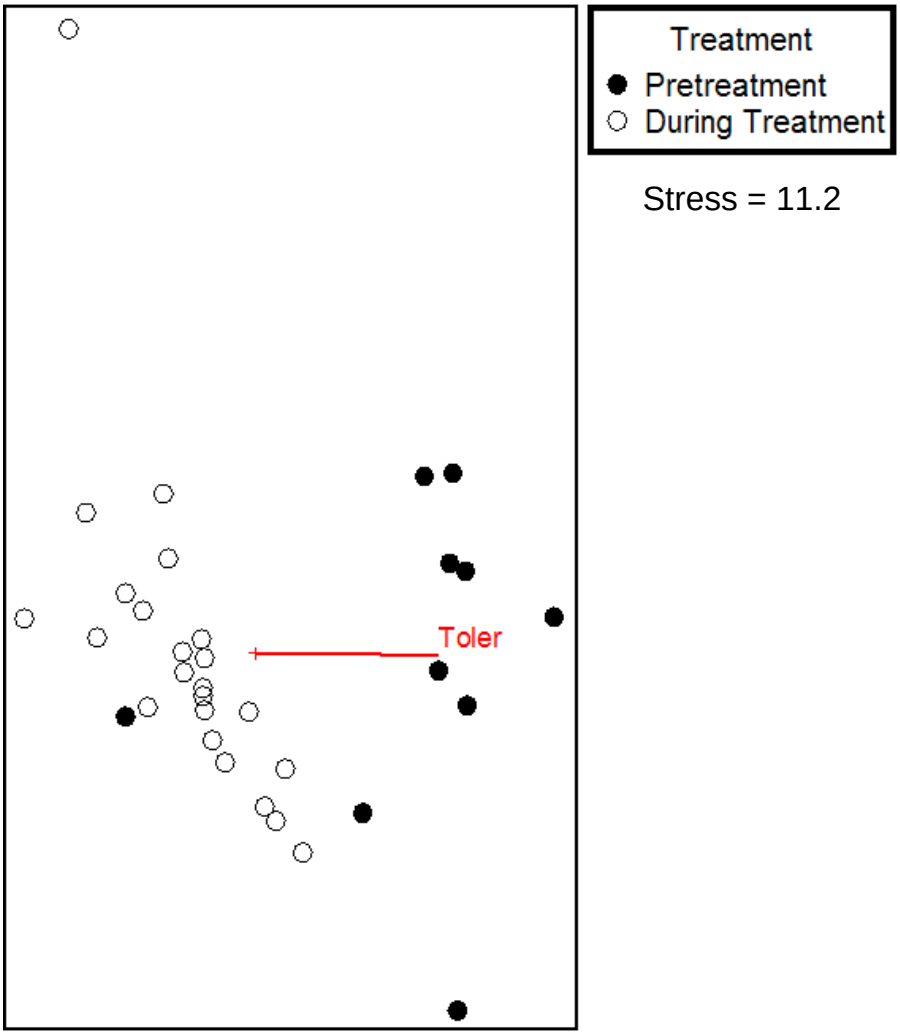

Figure 5. Non-metric Multidimensional Scaling ordination of Treatment Site 2 drift before and during treatment showing relationship of samples. The only joint plot with a $r^2 > 0.3$ was Toler (HBI tolerance values).

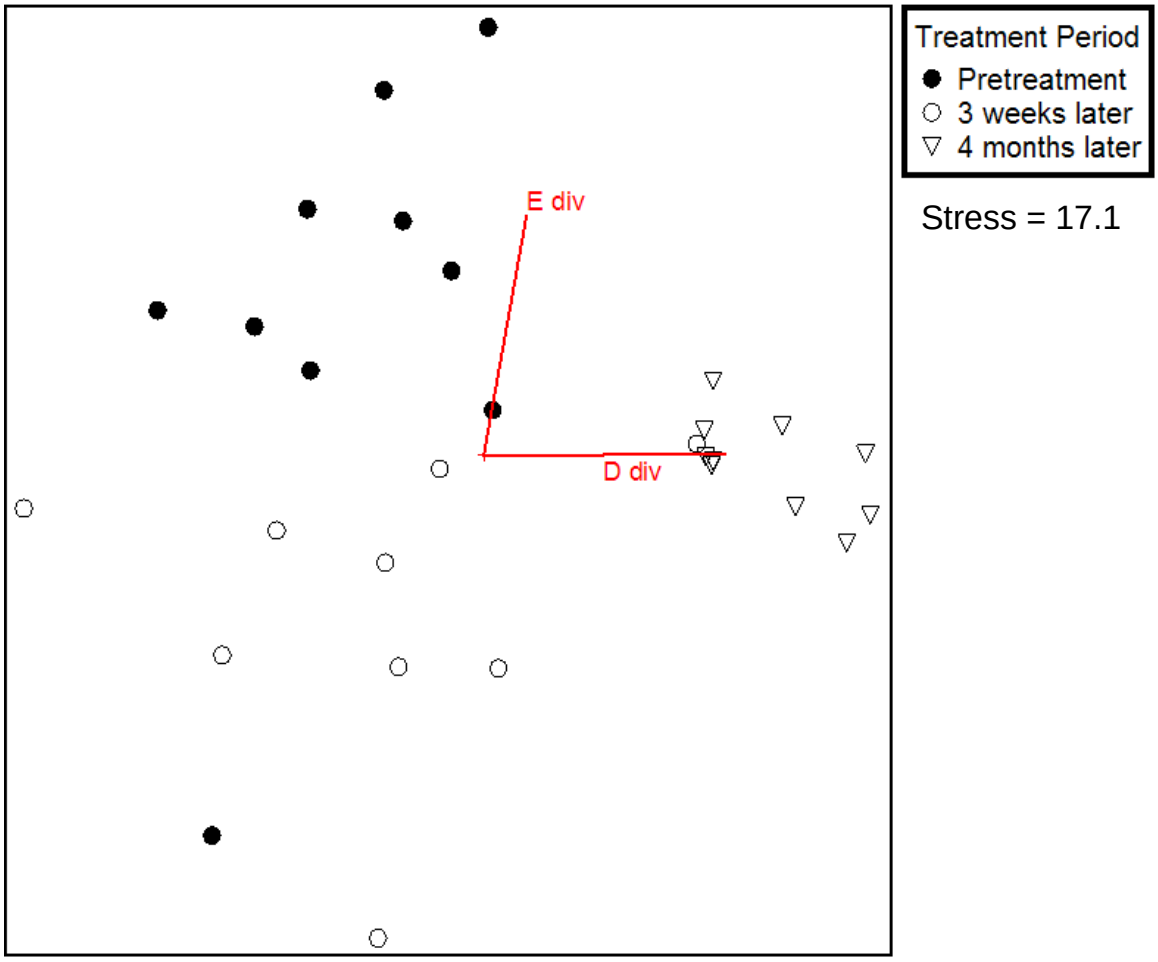

Figure 6. Non-Metric Multidimensional Scaling ordination of invertebrate riffle assemblages in Treatment Site 2 showing relationship of samples. Joint plots shown in red are variables that explained more that 40% variability ($r^2 > 0.4$) along axes 1 or 2. D div = Diptera Diversity, E div = Ephemeroptera diversity.

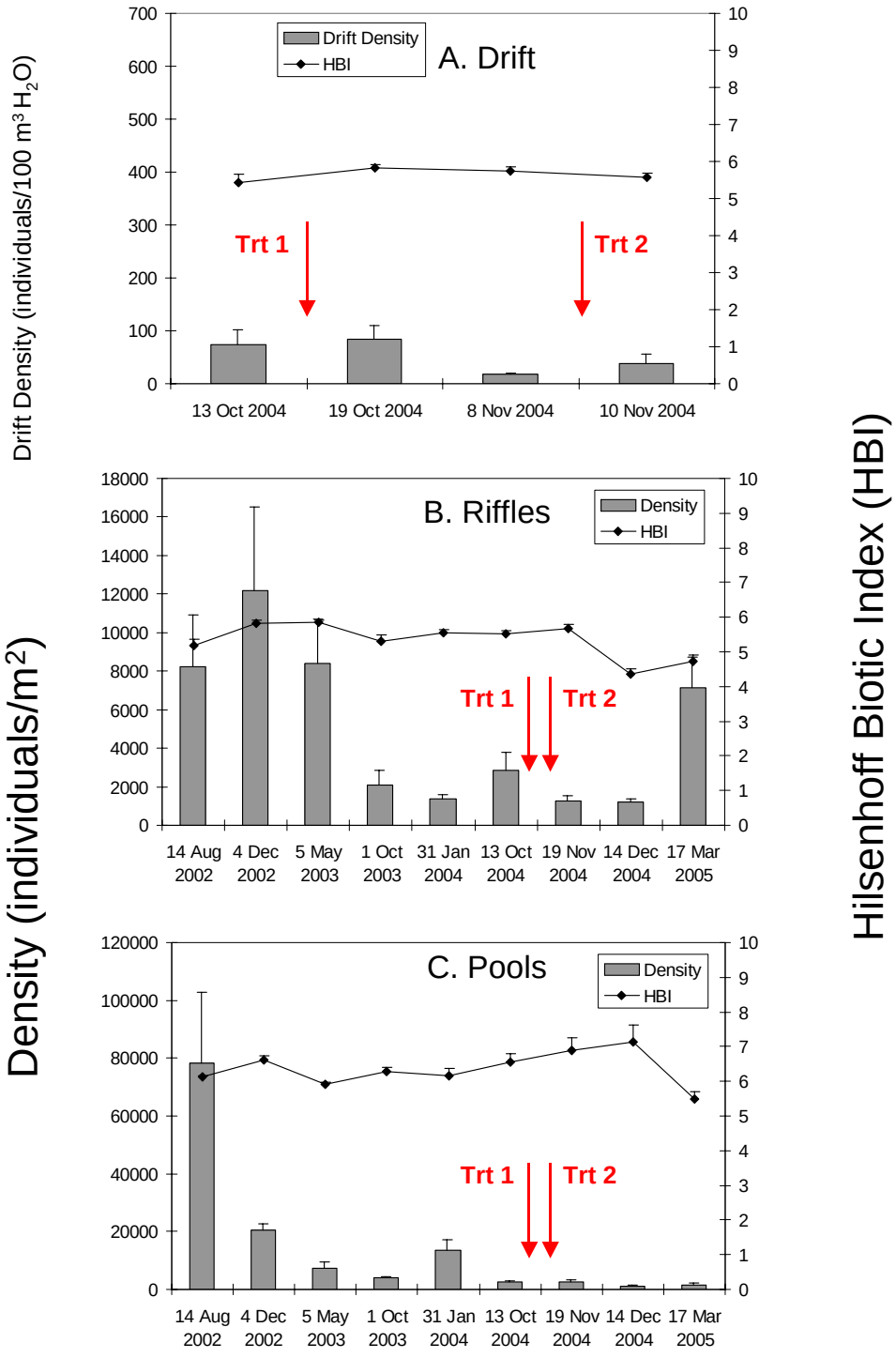

Figure 7. Mean drift, benthos densities and HBI in Control Site. A (top) is drift and HBI before and during treatments. B (middle) is densities and HBI in riffles before and after treatments. C (bottom) densities and HBI in pools before and after treatments. Treatments are indicated by red arrows. Bars = 1SE.

Evidence of rapid responses by macroinvertebrate communities to restored flow in a previously regulated river.

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Abstract

Although stream restoration programs are increasing, relatively few have associated monitoring programs to evaluate success of the project. Here, we studied the effects of flow restoration on the macroinvertebrates of Fossil Creek, a central Arizona stream that had flow diversion for the past 100+ years. Fossil Creek is a spring-fed, travertine stream with a small diversion dam scheduled for decommissioning. As part of a multi-faceted restoration project, flows were restored in June 2005. We collected 3 years of pre-restoration data and 15 months of post-restoration data. Our pre-restoration data suggested that patterns in travertine deposition were a major factor in determining macroinvertebrate assemblages. Following restoration, there was no detectable change in macroinvertebrate density or diversity in restored sites. However, macroinvertebrate assemblages in restored sites all showed significant shifts using non-metric multi-dimensional scaling – whereas there were no shifts in control sites. In particular, the site immediately below the dam shifted to resemble our control sites (relatively pristine, with no history of flow diversion) suggesting that increased flow is now an important determinant of assemblage composition. Other changes were driven by changes in densities of filter-feeding organisms, primarily *Hydropsyche* (Trichoptera: Hydropsychidae) and *Simulium* (Diptera: Simuliidae). We suggest that this is a response to changing flow and travertine deposition patterns associated with restoration. We conclude that macroinvertebrate communities can show rapid changes associated with stream restoration activities, despite a century of flow regulation. Long-term effects, however, will likely be a result of long-term trends in changing geomorphology and biogeochemistry associated with travertine deposition.

Introduction

Streams and rivers are dynamic ecosystems that recover quickly from disturbances through a self-cleaning property associated with unidirectional, constantly flushing flow (Hynes 1970). Hence, steams are widely considered to be systems with a high potential for restoration after short and long-term disturbances (Allan 1995) and successful restoration creates naturally resilient systems (Palmer et al. 2005). Restoration programs for streams have been increasing in recent years, and have taken many different forms (e.g. exotic species removal, clean water programs, riparian reparation, etc.). Despite this, few studies have reported on the success of stream restoration programs or have associated long-term monitoring to detect success (Niemi et al. 1990, Bernhardt et al. 2005). Over 1 billion dollars are spent annually on stream restoration projects in the US, with fewer than 10% having associated monitoring programs to document successes and failures (Bernhardt et al 2005).

Dams and the associated flow diversions are attractive subjects for stream restoration projects. The effects of dams and flow alterations on the aquatic communities are well understood (e.g. Power et al. 1996, Cortes et al. 1998, Vinson 2001, Greathouse et al. 2006). By altering physical parameters (especially flow) and disrupting the upstream/downstream connectivity of the river, invertebrate assemblages below dams often experience local extirpation of sensitive species and greatly reduced diversities (Vinson 2001, Greathouse et al. 2006). However, in the case of small diversion dams, these effects may be mitigated or erased by flow restoration. Rivers with smaller dams, which comprise the majority of dams in the United States, are candidates for restoration success for 2 primary reasons. First, the higher number and old age of many of them

make them likely sites to be decommissioned (Heinz Center 2002). Second, because ecological impacts associated with smaller dams are likely to be less severe than larger dams communities may be able to return to pre-disturbed states. (Thomson et al. 2005). Many of these small dams are run-of-the-river dams, meaning that excess flows discharge over the dams. The overflow helps maintain river connectivity and allows upstream allochthonous material to be supplied downstream, helping to maintain the natural processing of organic material, as in the River Continuum Concept (Vannote et al. 1980) and not the Serial Discontinuum Concept (Ward and Stanford 1983). Reservoir sizes behind these dams are smaller; increasing the likelihood that upstream migration of terrestrial stages of aquatic macroinvertebrates is uninhibited. Also, without a large reservoir there is no release of cold hypolimnetic water that often is responsible for large-scale changes in the aquatic macroinvertebrate assemblages (e.g. Vinson 2001). Finally, without the storage capacity of large reservoirs, the natural hydrograph is more likely to be maintained, although with water diversions the baseflow is typically greatly reduced.

In this study, we report on the effects of flow restoration to aquatic invertebrates in a travertine forming, central Arizona stream that had water diverted for the past 100 years. A small dam (6 m tall) had diverted water into a flume network for hydropower generation at a series of 2 small, downstream generating plants. The diversion had large impacts not only on the flow regime of the river, but radically changed the geomorphology of the river by redistributing the deposition of travertine in the stream (Malusa et al. 2003, Marks et al. 2006). Travertine has major effects on invertebrate assemblages (Minckley 1963), and the historic redistributing of travertine presumably altered the invertebrate assemblages. In June 2005 flow was restored throughout Fossil

Creek as part of a dam decommissioning. The diversion dam was left in place and will likely be lowered or removed in 2009. This decoupling of flow restoration and dam removal allowed us to independently study the effects of increased flow on macroinvertebrate communities.

In response to flow restoration, we expected to see short-term increases (just over 1 yr) in diversity but no change in densities at restored sites. Our reasoning for this is based on several factors. First, although there may be initial decreases in both diversity and density caused by the disturbance of restoration activities we expect this to be short-lived (weeks to months) because of rapid colonization. Southwest invertebrates are typically multi-voltine, having many generations in a single year – often as quick as two wks for *Baetis* sp. (Ephemeroptera: Baetidae) (Gray 1981), a prevalent mayfly in Fossil Creek. Hence, any disturbance-caused declines could be quickly overcome with surviving colonists from affected sites, or from new colonists in unaffected upstream sites. Second, because there is no sediment plug behind a dam associated with flow restoration, we do not expect any large smothering or sediment pollution associated with this particular event. Third, flow restoration will create a large amount of new habitat – increasing habitat availability, allowing for new microhabitats, and affording opportunity for new species of macroinvertebrates to colonize newly created habitats. We expect the new habitat to increase the overall number of invertebrates, but individuals per square area should be unaffected. Lastly, increased connectivity of the headwaters with the lower reaches should assist in downstream transport of invertebrates and resources – which should help increase diversity. However, we also expect species composition to

change as a result of increased nutrient supply, changing flow conditions, and increased downstream sediment transport from the headwater reach following restoration.

We expect long-term effects (5+ years) of restoration to be manifested not only by the above short-term effects, but also by the changing travertine geomorphology. The redistribution of travertine in Fossil Creek should reinforce and enlarge the travertine dams immediately below the diversion dam, and increase the distribution of travertine dams. Previous locations that may have been characterized by cobble substrate may be transformed into travertine dam/pools, and hence alter the macroinvertebrate habitat and assemblage.

Methods

Study Site

Fossil Creek is a perennial, travertine depositing spring-fed stream originating from a layer of Mississippian Naco Limestone along the Mogollon rim in central Arizona (Fig. 1). Baseflow from the springs are typically 1,218 L s^{-1}, but the majority of the water was diverted into a flume at the dam, less than 1 km from the spring source. At a powerplant mid-way down the stream, a portion of the water was returned, creating 3 distinct flow regimes: 1) unaltered, full flow above the diversion dam, 2) seepage flows of about 5.6 L s^{-1} below the diversion dam, and 3) increased flows of 56.6 L s^{-1} just below the mid-way power plant.

We choose 6 sampling sites prior to restoration to encompass the range of flow and travertine deposition (Table 1). Two of these were above the dam upstream of the small reservoir, 2 were in the seepage zone, and the last 2 were below the power plant where 56.6 L s^{-1} was returned to the streambed. Despite high concentrations of Ca^{2+} and

HCO_3^-, the sites above the diversion dam (Control Sites 1 and 2) do not exhibit travertine deposition because they are supersaturated with CO_2. The relatively low gradient and mostly laminar flow of the control sites limit the off-gassing of CO_2. Below the diversion dam (Restored Site 1), travertine deposition began and created a series of small travertine dams (Fig. 2), creating a travertine dam/pool series where the dams formed steep, shallow cascades. This section was fed by seepage flows, and at the point just above the mid-way power plant, much of the Ca^{2+} and HCO_3^- had been deposited as $CaCO_3$ so that there was just a slight amount of travertine deposition (Restored Site 2). Below the power plant, a portion of flume water (~56.6 L s^{-1}) was returned to the stream channel after being used to generate power. This return of water introduced a new source of water supersaturated with respect to $CaCO_3$ renewed the deposition of travertine, and created a new series of travertine dams (Restored Site 3). By the time this water had reached the furthest downstream site (Restored Site 4), $CaCO_3$ supersaturation levels had again dropped so that substrate was armored by low rates of travertine precipitation which creates coatings but there was no travertine dam formation.

Fossil Creek Restoration

A collaborative effort of many government and non-government agencies (e.g. Fish and Wildlife Service, National Forest Service, Arizona Game and Fish, Bureau of Reclamation, Arizona Public Service, and Northern Arizona University) pursued the restoration of Fossil Creek in 2 primary ways: 1) Fish repatriation and 2) Flow restoration.

Prior to flow restoration, fish repatriation was conducted in the fall of 2004 by installing a fish barrier in the lower reaches of Fossil Creek, salvaging available native

fish, eradicating non-natives with the piscicide antimycin A, and reintroducing the native fish. Effects of the antimycin A treatments on aquatic invertebrates are covered in detail elsewhere (Dinger and Marks, in revision), but was characterized by short term decreases in density and diversity at affected sites, although invertebrates densities had recovered within 5 months, and diversity was increasing by March 2005 (Dinger and Marks, in revision).

Flow into the flume was shut off on 18 June 2005, and full flow was returned to Fossil Creek.

Invertebrate sampling

Invertebrates were haphazardly sampled with 5 replicate Surber samples (250 μm mesh size, 0.093 m^2) at each sampling site and period. Substrate was agitated and scrubbed, and in the case of travertine areas, a screwdriver was used to assist in the breakup of armored substrate. Agitation and scrubbing was continued until all substrate was affected. Samples were transferred to a 5 g bucket, and elutriated to separate invertebrates from inorganic substrate. The remaining substrate was scanned for snails and stone-cased Trichoptera. Samples were then filtered through a fine-aquarium net (100 μm), and preserved in 95% ethanol.

Invertebrates were sorted from associated debris and detritus under low power magnification, and enumerated and identified to the lowest practical level (except Chironomidae which was left at family) using standard taxonomic references (e.g. Pennak 1989, Thorp and Covich 1991, Merritt and Cummins 1996, Wiggins 1996).

Invertebrate collections for this study started in August 2002 and continued in December 2002, May 2003, October 2003, January 2004, March 2005, September 2005,

and August 2006. During the fall and winter of 2004, we focused sampling efforts on examining the effects of the fish renovation project with antimycin A (Dinger and Marks, in revision).

Data analyses

Non-metric Multidimensional Scaling (NMS) ordinations were used to examine patterns and shifts in invertebrate assemblages. Invertebrate densities were standardized to species maximum to equalize the importance of each taxon, and converted to a similarity matrix using the Bray-Curtis similarity index in the computer program PRIMER 5 (version 5.2.8) prior to NMS ordination. Significance of grouping was then tested using the ANOSIM routine in PRIMER. ANOSIM is a permutation-based hypothesis test that tests for differences between *a priori* groups using Bray-Curtis dissimilarity measures. In ANOSIM, the R-value is a measure of grouping strength, with a value of 1 indicating strong separation and a value of 0 indicating no separation. Where there were significant differences, we used the SIMPER routine to examine the species contributing to the observed group differences. This routine calculates the percent contribution of each species to the dissimilarity observed between the groups. Lastly, to understand which species are driving overall patterns, we used the PRIMER routine BVSTEP, which is a step wise procedure based on rank correlations to find the most "influential" species driving observed patterns in ordinations. Taken together, these 4 analyses offer a robust method of examining ecological data.

Effects of flow restoration on invertebrate densities (log x +1 transformed) and species richness were analyzed using a Before-After/Control-Impact Analysis of Variance (BACI ANOVA). In this model, sites above the dam served as control sites

unaffected by flow restoration, whereas sites below the dam were impact sites. The significance of flow restoration is determined by the significance of the interaction term (Green 1979).

Results

Data from pre-restoration samples showed that there were distinct groupings based on the amount of travertine deposition (Fig 3.) ANOSIM analysis showed that all groups were significantly different. Sites with no travertine (Control Sites 1 and 2) were significantly different from both travertine dam sites (Restored Sites 1 and 3) ($R = 0.599$, $p = 0.001$) and from moderate travertine ($R = 0.503$, $p = 0.001$). Sites with moderate amounts of travertine were significantly different from sites with travertine dams ($p = 0.001$), but the effect size ($R = 0.07$) was small, indicating substantial overlap in these assemblages.

Our pre-restoration data did indicate that downstream sites (to be Restored Sites) had similar levels of density and diversity as pristine, upstream sites (Control Sites). Following restoration, there was no discernable pattern on either density or species richness, in either the Control or Restored Sites (Fig. 4). There was a significant effect of date (before vs. after) on the density data, but the test for flow restoration effects using the interaction term was non-significant (Table 2).

Following our predictions, all of the restored sites had significantly different invertebrate assemblages following flow restoration (Table 3). Likewise, the control sites did not show any shift in species assemblage (Table 3, Fig. 4). Using R-values as a measure of strength of change, the site that changed the most was the Restored Site 1 with a value of 0.455, with the ordination showing distinct grouping (Fig. 5). Other sites

showed interesting patterns, such as the Restored Site 2, which had distinct post-restoration grouping, but had one set of samples (September 2005) at the right side of the graph, and August 2006 samples forming another distinct group on the left side, with pre-restoration samples in between (Fig. 6). As shown using the ANOSIM R-values, the changes in the last 2 Restored Sites (3 and 4) were not as strong, but still distinct (Fig. 7).

The species responsible for observed differences using SIMPER were generally the same in most cases: *Simulium* (Diptera: Simuliidae), *Hydropsyche* (Trichoptera: Hydropsychidae), *Baetis* sp., Chironomidae (Diptera) (Table 2). However, the direction of change in these species was not consistent among sites: *Simulium* decreased in the Restored Sites 1 and 2, but increased in the Restored Site 3. Similarly, *Hydropsyche* decreased in the Restored Site 3, but increased Restored Site 4.

To understand the context of the changing downstream sites, we performed a follow-up ordination using all sampling sites. To reduce noise in the ordination (of 240 samples), we composited the 5 replicate surber samples from each date into a single, composite sample. This ordination shows the similarity of the Control sites, pre and post restoration, and highlights the differences of the other sites (Fig. 6). In particular, the site directly below the dam (Restored Site 1) has shifted post-restoration to resemble the Control Sites, towards the left side of the plot. The other sites, however, were shifted to the right. Using BVSTEP, 5 taxa were the most influential for the ordination pattern in Figure 8 – Chironomidae, *Baetis*, Elmidae (Coleoptera), *Simulium,* and *Thraulodes* (Ephemeroptera: Leptophlebiidae).

Discussion

The aquatic macroinvertebrates of Fossil Creek responded rapidly to flow restoration, although there was no change in either average densities or diversity. Instead, the response was made of species shifts in restored sites. Although diversity in Fossil Creek was not increased by flow restoration, other studies on diversity have yielded mixed results. Some studies show higher diversity in non-regulated reaches relative to regulated reaches (Munn and Brusven 1991) whereas others suggest that there is no relationship between diversity and the degree of regulation or number of diversions (e.g. Collier 2002, Marchant and Hehir 2002). Our results show that despite shifts in community composition, diversity did not increase as predicted with restoration of flows. This is consistent however, with our pre-restoration observations where neither diversity nor abundance in the relatively pristine sites above the diversion dam were higher relative to the disturbed sites. During several sampling periods, the highest diversity was found in the low flow, travertine dam sites. Since diversity was already high in these regulated sites, it is not surprising that flow regulation did not, as yet, further increase diversity in the restored sites.

In just slightly more than a year after flow restoration, the invertebrate assemblages in Fossil Creek are already showing significant responses. Firstly, the site immediately below the dam started to resemble the unchanged sites above the dam. The puzzling aspect is that our perception of the dominant substrate in this reach has not changed – travertine dams are still actively forming. Our pre-restoration data had demonstrated strong differences in invertebrate assemblages depending on travertine deposition (Fig. 3). Hence, we suggest that in this site, the controlling factor of

macroinvertebrates shifted: from travertine towards flow. This could be caused by 2 factors: 1) Increased connectivity has increased upstream invertebrates dispersal, allowing them to inhabit previously unavailable lower reaches, and/or 2) the importance of flow in determining assemblages now outweighs the import of travertine. The effects of flow on aquatic invertebrates are complex, but well studied (Hynes 1970, Allan 1995). Effects of flow on the availability of invertebrate habitat (e.g. Minshall 1984), flow effects on primary production (e.g. Keithan and Lowe 1985), nutrient supply, evolutionary adaptations (e.g. Hynes 1970), feeding mechanisms (Cummins 1974), microdistribution of invertebrates (Statzner 1981), dispersal ability of invertebrates (Elliott 1971) etc. have all been studied, but the effects of travertine on invertebrates are still poorly studied (Minshall 1984).

Secondly, invertebrates downstream also appear to be changing in response to flow restoration, a shift that appears to be mostly made up of changing densities of *Simulium* and Chironomidae midges. Two downstream sites were also marked by an increase in *Hydropsyche* caddisflies densities, (Restored Site 1 and 4). Increases in *Simulium* were often offset by decreases in *Hydropsyche,* and vice versa. These 2 taxa compete for the same space and resources (Hemphill and Cooper 1983, Hemphill 1988). We hypothesize that the changes in the 2 taxa are due to interacting effects of changes in travertine deposition and flow. Our observation prior to flow restoration is that *Hydropsyche* benefited from travertine deposition in reinforcement of their silken capture nets, used for filter-feeding. This reinforcement would help the net to withstand increased velocities, considering that Hydropsychidae caddisflies are commonly relegated to slower velocities (Hemphill and Cooper, 1983). Increased flows, along with

reduced travertine deposition might give *Simulium* larvae the advantage in this situation – especially since *Simulium* is the more competitive in higher flows (Hemphill 1988). In the site with increased flows, but also increasing travertine, the Restored Site 1, *Hydropsyche* became more dominant. In the site with increased flows, but decreased travertine (the Below Power Plant site), *Simulium* became the more abundant. Both of these observations are consistent with our suggestion that both flow and travertine are determining the outcomes of *Hydropsyche* and *Simulium* competition.

Changes in flow might be particularly important to both *Simulium* and *Hydropsyche* since they are filter-feeders, requiring flow to transport processed detritus downstream to lower river habitats (Cummins 1974). As predicted by the River Continuum Concept (Vannote et al. 1980), re-establishing connectivity between headwaters and lower stream reaches would result in increasing fine-particulate organic matter downstream. This is despite the continued presence of reservoir behind the diversion dam, which still acts to trap much of the suspend sediments prior to overflow. Reestablishing this connectivity is especially relevant because one of the few large shredding organisms of Fossil Creek, the caddisfly *Phylloicus* sp. (Trichoptera: Calamoceratidae) is abundant in the Control Sites (although infrequently represented in our samples owing to a preferred habitat in unsampled pools and side channels). Hence, restoring flow connectivity between the small headwater reach (less than 1 km) and downstream sites increased available resources for filter-feeders downstream.

Lastly, we did not observe any decrease in invertebrate density following flow restoration. In the available flow restoration literature, a common theme is that both invertebrate diversity and density decrease following restored flows (e.g. Bednarek

2001). The important difference is that in all of these examples, the dam was also removed as part of stream restoration – releasing the reservoir sediment plug. This follows the findings of Thomson et al. (2005) who observed that densities declined only with the removal of a Pennsylvania dam, and not with an initial partial removal. In Fossil Creek the dam has so far been left intact, but managers will likely remove a portion or all of it. Hence, the potential for sediment to temporarily decrease the invertebrate densities and diversity still exists for Fossil Creek, although dam removal studies have shown this decrease to be short-lived (Bednarek 2001, Stanely and Doyle 2003), the process of leaching stored nutrients and transporting sediments out of the system could last a number of years (Ahern and Dahlgren 2005).

Long-term changes to the invertebrates will also depend on the changing geomorphology of Fossil Creek. Even though the amount of travertine depositing in different reaches has been altered, the relics of 100+ years of flow alteration have not changed. For example, in the Restored Site 3 reach there are still large, remnant dams, although they are no longer forming as they used to. In time, these may break down due to flooding or other disturbance, eventually restoring the original, pre-dam geomorphology, effectively changing the invertebrate habitat. Future changes will also depend on the whole or partial removal of the diversion dam. Currently the reservoir behind the dam still serves as a sediment trap, allowing suspended sediments to settle. Although we believe that the restoration of flows have so far allowed for an increase in downstream transport of these particles, removal of the dam will further enhance food resources for downstream filter-feeders.

These results show that aquatic communities can respond quickly to restoration. Although it is too early to document the long term changes caused by restoration this data shows how responsive macroinvertebrates can be. Depending on both invertebrate colonization rates and the changing travertine geomorphology of Fossil Creek, it will require long-term (5+ years) of monitoring until the effects are known.

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Table 1. Sample site characteristics and hypothesized changes for invertebrate assemblages in Fossil Creek, Arizona. Note that after restoration flows are 1,218 L s^{-1} for all sites.

Site	Approx. Distance Downstream (km)	Flows before Restoration (L s-1)	Travertine Deposition Pre Restoration	Predicted Travertine Deposition Post Restoration	Change Predicted?
Control Site 1	0	1,218	None	None	No
Control Site 2	0.25	1,218	None	None	No
Restored Site 1	0.5	5.6	Active Dams	Active Dams	Yes
Restored Site 2	6.5	5.6	Slight	Active Dams	Yes
Restored Site 3	7	56.6	Active Dams	Armoring	Yes
Restored Site 4	16	56.6	Armoring	Slight	Yes

Table 2. BACI ANOVA results for density and species richness. *p*-values in bold are significant at $\alpha = 0.05$.

		Density			Species Richness		
	df	Mean SS	F Ratio	*p*-value	Mean SS	F Ratio	*p*-value
Date (i.e. Before/After)	1	1.636	6.8552	**0.0095**	0.571	0.0265	0.8708
Location (i.e. Above/Below)	1	0.564	2.3612	0.1259	25.459	1.1801	0.2786
Date X Location	1	0.500	2.0961	0.1492	60.240	2.7923	0.0962
Residual	206	0.239			21.574		

Table 3. ANOSIM, SIMPER and BVSTEP results for pre to post-flow restoration changes. % contribution to dissimilarity is the amount that species contributed to the sites being different. *p*-values in bold are significant at $\alpha = 0.05$.

	ANOSIM Results		SIMPER Results			BVSTEP Results	
Site	R Value	*p*-value	Species contributing to dissimilarity	% Contribution	Post restoration direction of change	Rank correlation	Species contributing to pattern
Control Site 1	0.052	0.24	N/A				N/A
Control Site 2	0.035	0.302	N/A				N/A
Restored Site 1	0.455	0.001	Chironomidae	23.7	↓	0.968	*Baetis* sp.
			Simulium sp.	19.3	↓		*Simulium* sp.
			Baetis sp.	17.2	↓		Chironomidae
			Culicoides sp.	7.9	↑		*Culicoides* sp.
Restored Site 2	0.255	0.006	Chironomidae	29.4	↓	0.980	Chironomidae
			Baetis sp.	24.5	↓		*Baetis* sp.
			Simulium sp.	21.7	↓		*Simulium* sp.
			Oligochaete	4.4	↓		
Restored Site 3	0.182	0.009	*Simulium* sp.	34.1	↑	0.975	Chironomidae
			Chironomidae	21.8	↔		*Simulium* sp.
			Hydropsyche sp.	15.4	↓		*Hydropsyche* sp.
			Baetis sp.	8.6	↓		
Restored Site 4	0.218	0.009	Chironomidae	19.9	↓	0.966	Chironomidae
			Baetis sp.	19.1	↓		*Baetis* sp.
			Hydropsyche sp.	18.1	↑		*Baetodes* sp.
			Simulium sp.	14.2	↔		*Simulim* sp.

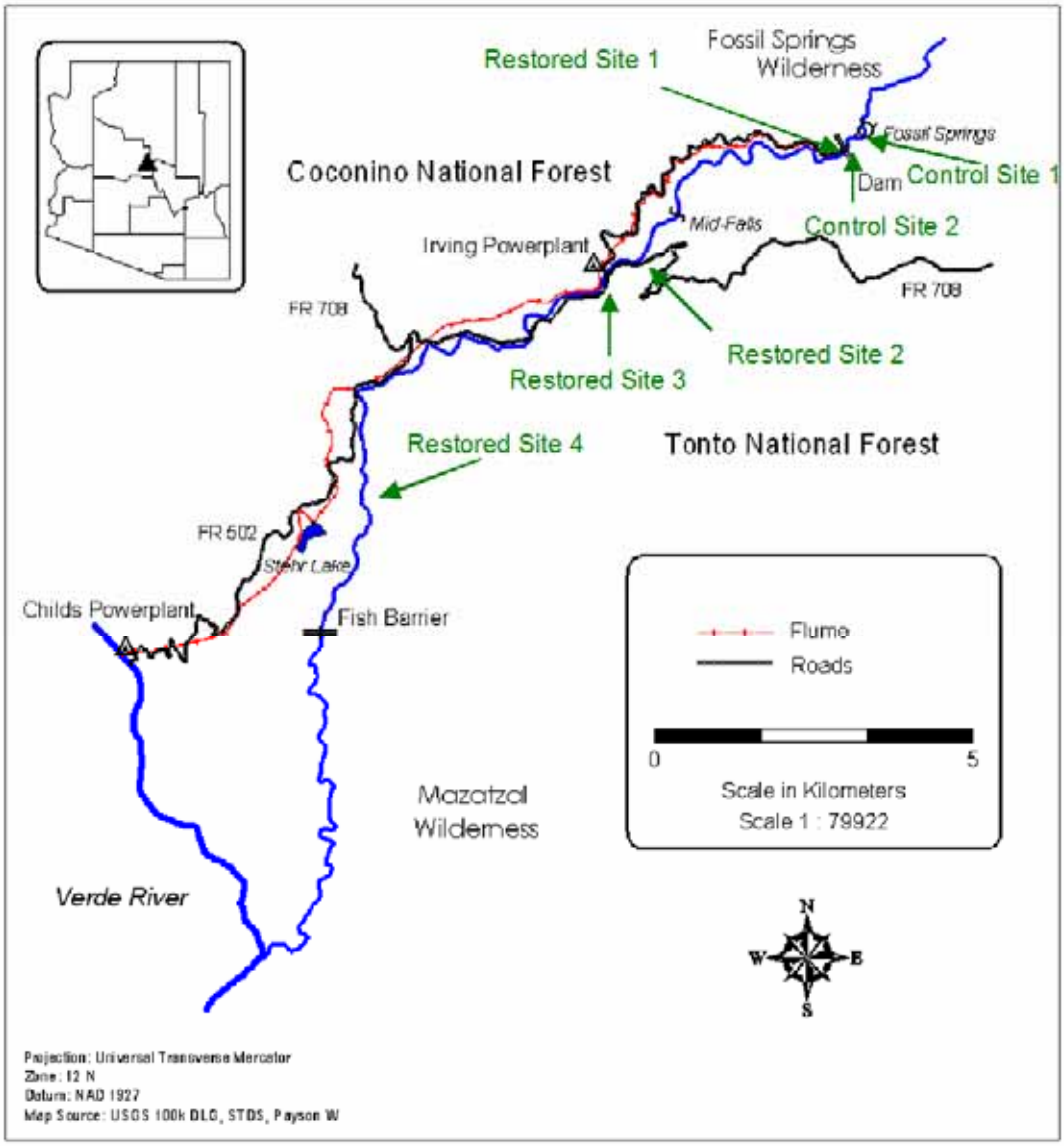

Figure 1. Map showing physical and cultural aspects of the Fossil Creek watershed. Green arrows indicate sampling site locations

Figure 2. Travertine dams forming dam/pool series in the Restored Site 1, taken before restoration.

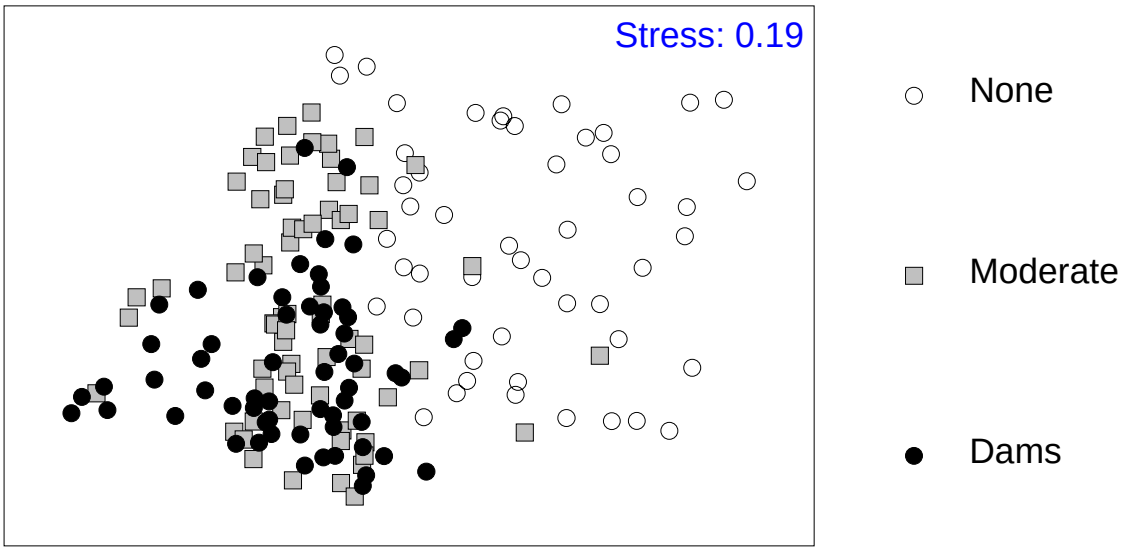

Figure 3. NMS ordination of pre-restoration data showing influence of travertine on invertebrate assemblages. Symbols indicate amount of travertine deposition for each sample: None = Control Sites 1 and 2, Moderate = Restored Sites 2 and 4, Dams = Restored Sites 1 and 3.

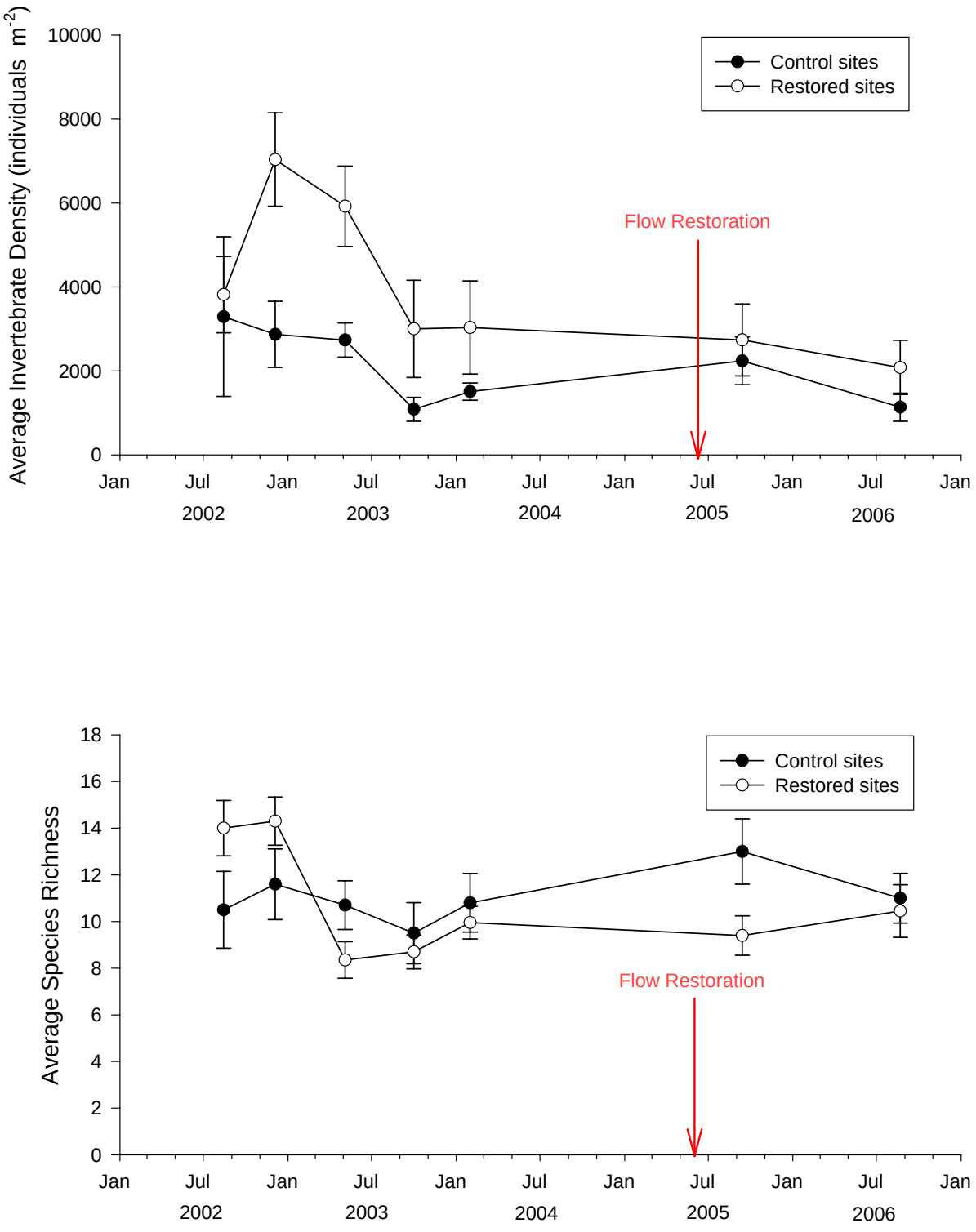

Figure 4. Average aquatic invertebrate densities (Top) and Species Richness (Bottom) for Control sites (Black circles) and Restored sites (white circles). Red line indicates date of flow restoration

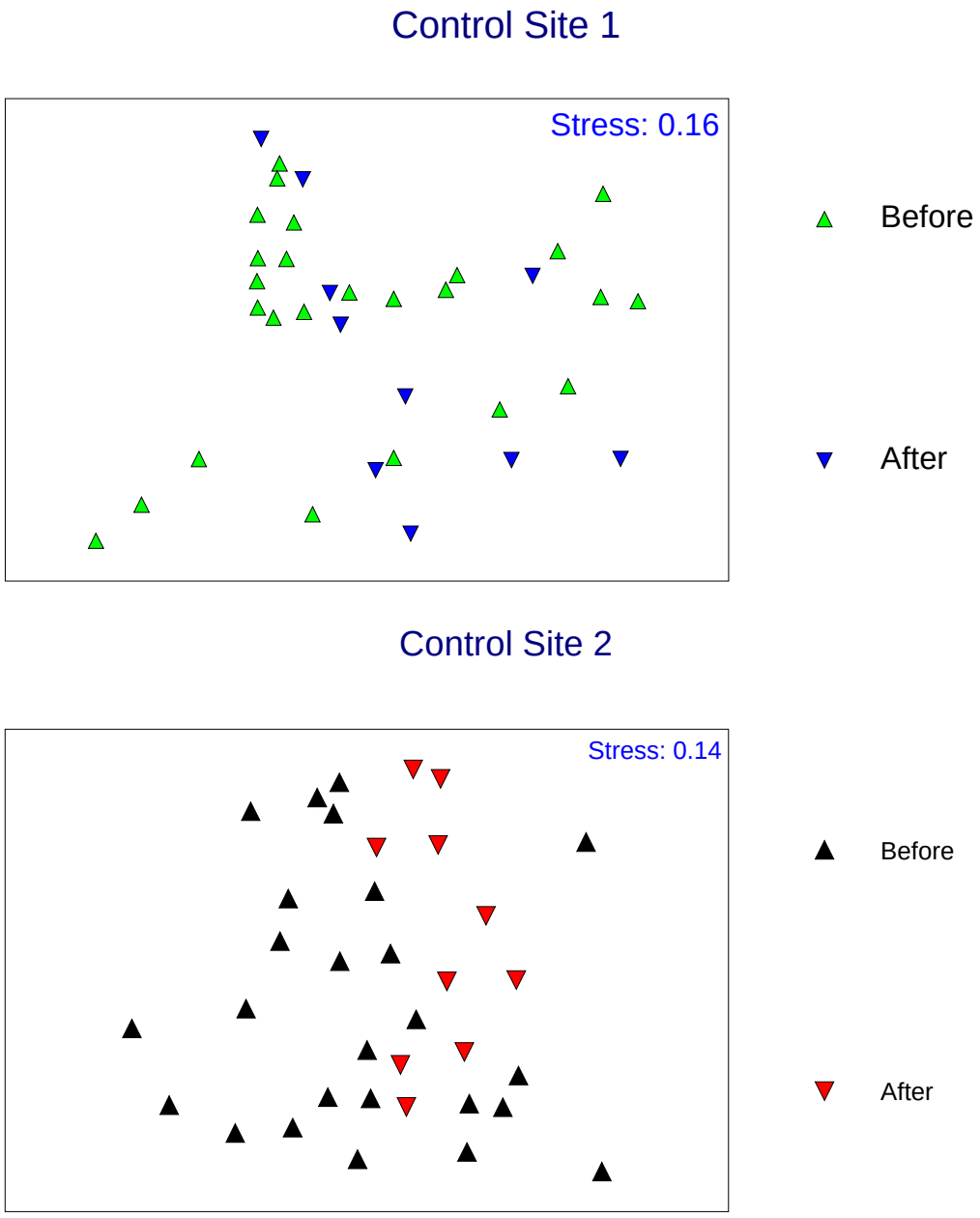

Figure 5. MDS ordinations of Control Site 1 (Top) and Control Site 2 (Bottom) invertebrate assemblages. ANOSIM analyses did not reveal significant differences following restoration at either of these sites.

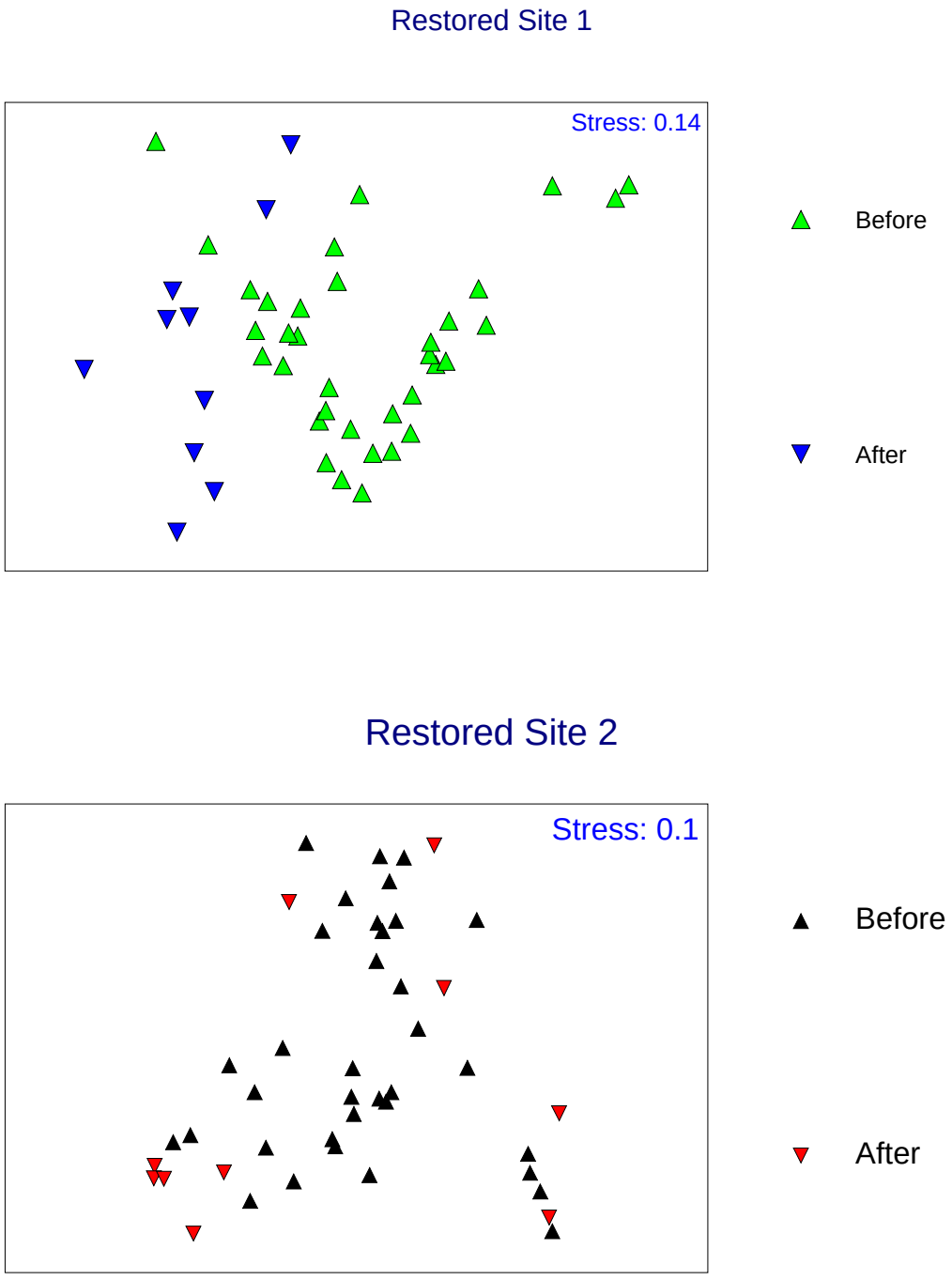

Figure 6. MDS ordinations of Restored Site 1 (Top) and Restored Site 2 (Bottom) invertebrate assemblages. ANOSIM analyses indicate significantly different invertebrate assemblages before and after flow restoration.

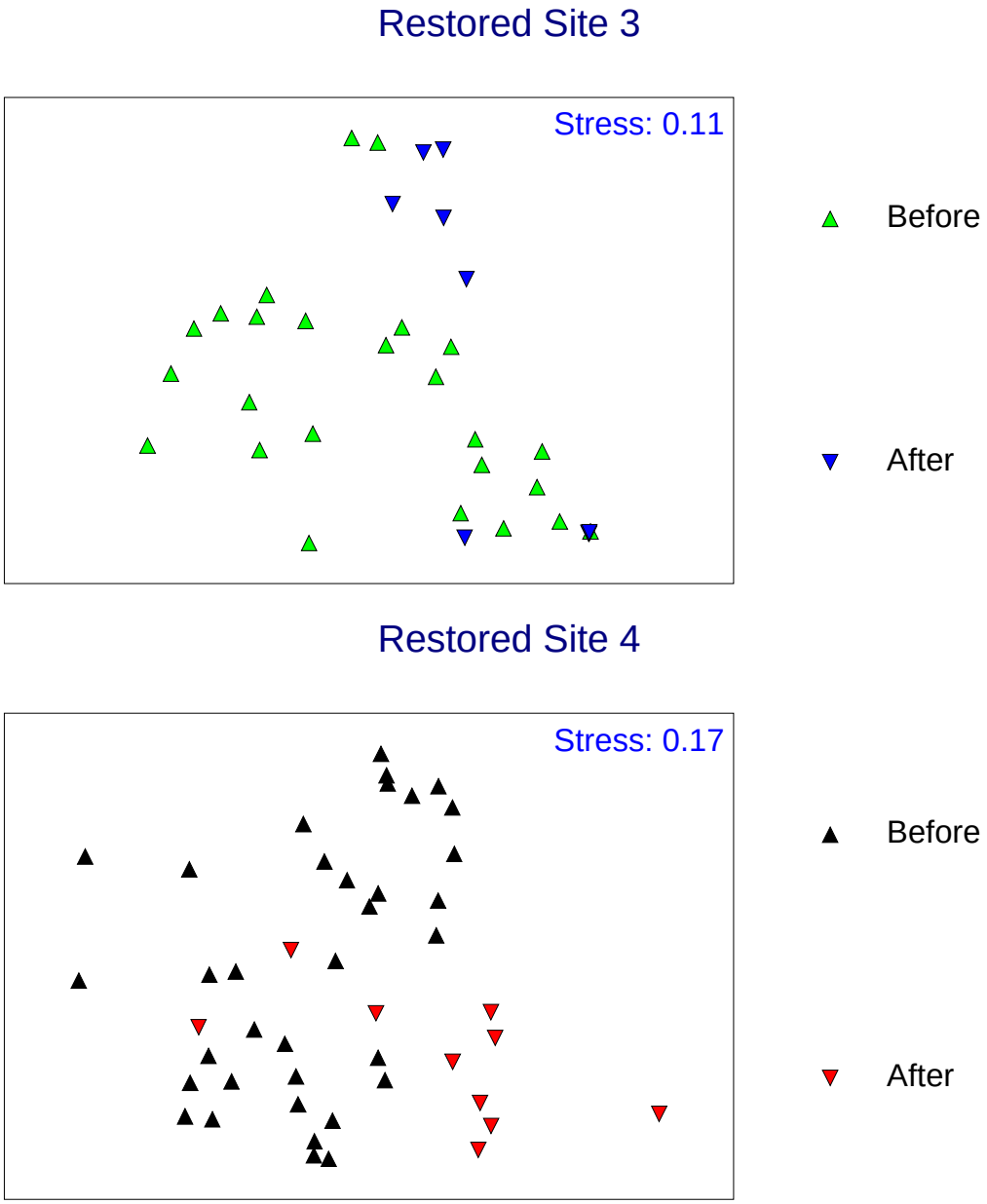

Figure 7. MDS ordinations of Restored Site 3 (Top) and Restored Site 4 (Bottom) invertebrate assemblages. ANOSIM analyses indicate significantly different invertebrate assemblages before and after flow restoration.

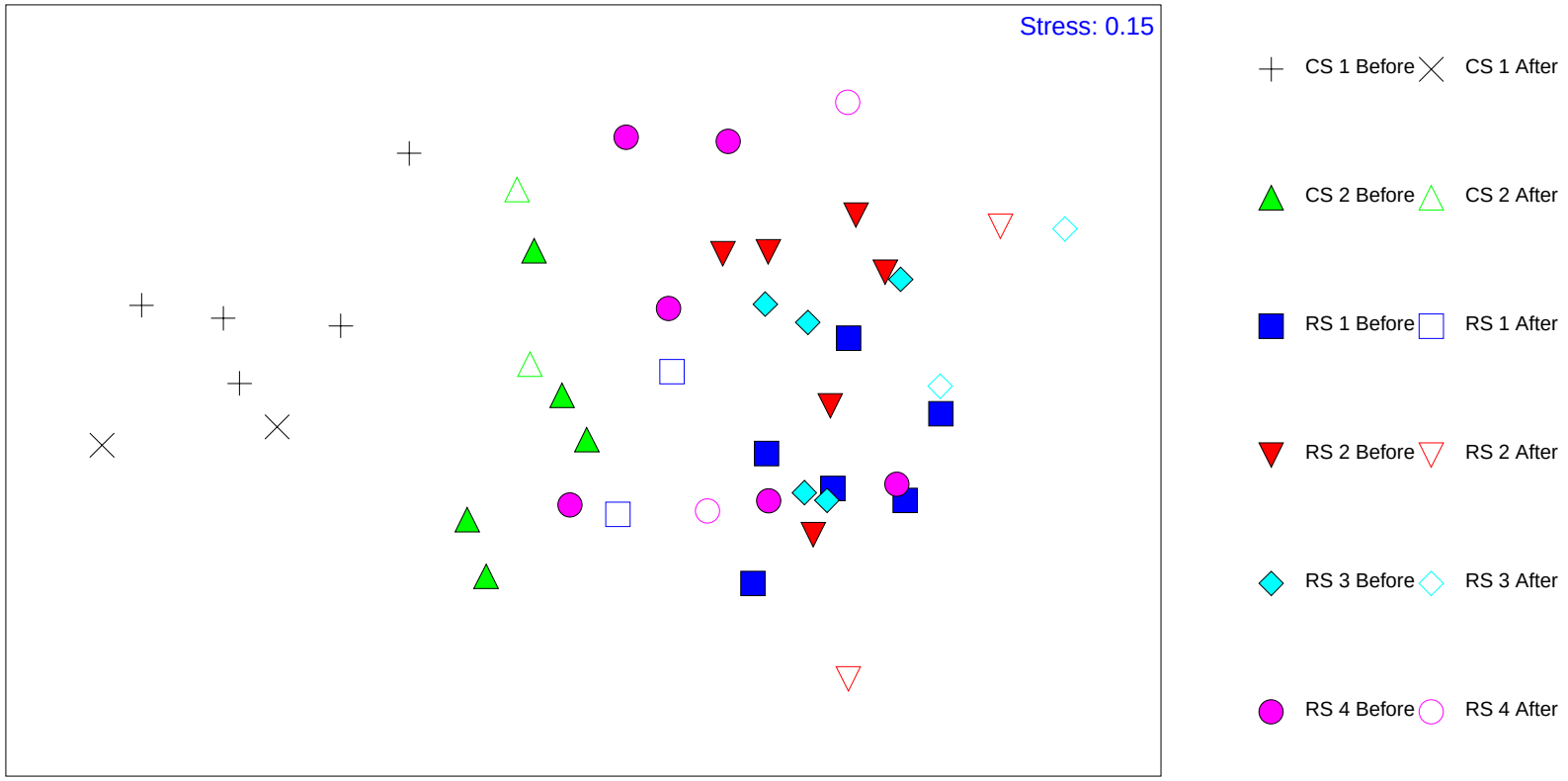

Figure 8. NMS ordination of composited samples for each sampling date. CS = Control Sites, RS = Restored Sites.

Appendix 1. Taxa list and numbers collected in all 6 sites over the course of the study.

			Control Site 1		Control Site 2		Restored Site 1		Restored Site 2		Restored Site 3		Restored Site 4	
Order	Family	Genus species	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Amphipoda	Hyalellidae	*Hyalella*	278	107	0	0	0	0	0	0	1	0	0	0
Annelida	Hirudinea	Unknown	8	2	0	0	0	0	0	0	0	0	0	0
Annelida	Tubificidae	*Branchiura sowerbyi*	0	0	2	0	0	0	125	0	68	3	79	2
Annelida	Oligochaete	Unknown	373	310	152	25	260	109	620	16	171	207	155	45
Bivalvia	Corbiculidae	*Corbicula*	0	0	0	0	0	0	0	1	1	0	2	0
Bivalvia	Pisiidae	*Pisidium*	11	1	21	0	10	0	2	0	2	0	2	0
Coleoptera	Curclionidae	Unknown	0	0	0	0	0	0	0	0	1	0	1	0
Coleoptera	Dryopidae	*Helichus*	8	2	1	1	0	0	5	0	1	0	0	0
Coleoptera	Dryopidae	*Postelichius*	6	4	0	0	10	6	37	1	5	1	5	1
Coleoptera	Dyticidae	nr. *Agabetes*	0	0	0	0	2	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	*Stictotarsus*	6	0	0	0	9	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	Unknown	0	4	0	1	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	*Neoclypeodytes*	0	4	0	0	0	0	0	0	0	0	0	0
Coleoptera	Elmidae	Various	1020	704	598	93	136	134	432	10	102	42	198	30
Coleoptera	Haliplidae	*Peltodytes*	0	2	0	0	0	0	0	0	0	0	0	0
Coleoptera	Haliplidae	*Haliplus*	0	0	0	0	2	0	0	0	0	0	0	0
Coleoptera	Hydrophilidae	*Berosus*	0	2	0	1	0	0	3	0	0	0	0	0
Coleoptera	Hydrophilidae	*Laccobius*	0	0	0	0	0	0	0	0	0	0	1	0
Coleoptera	Hydrophilidae	*Tropisternus*	0	0	1	0	1	0	0	0	0	0	0	0
Coleoptera	Lutrochidae	*Lutrochus*	0	0	0	0	0	0	4	0	0	0	96	14
Coleoptera	Psephenidae	*Psephenus*	0	0	2	1	0	0	0	0	0	0	0	0
Coleoptera	Scirtidae	*Scirtes*	1	0	0	0	0	0	0	0	0	0	0	0
Coleoptera	Staphylinidae	Unknown	0	1	0	0	0	1	0	0	0	0	0	1
Collembola	Unknown	Unknown	0	0	0	0	0	0	0	0	0	0	1	0
Decapoda	Cambaridae	*Orconectes*	0	0	0	0	0	0	0	0	0	1	0	1
Diptera	Ceratopogonidae	*Bezzia*	7	2	20	1	54	4	19	2	4	11	12	0
Diptera	Ceratopogonidae	*Culicoides*	7	2	4	6	92	105	65	42	56	24	12	2
Diptera	Ceratopogonidae	*Forciomyia*	0	0	0	0	0	0	0	0	1	0	0	0
Diptera	Chironomidae	Various	1198	125	1813	242	15683	869	10529	695	2508	876	2381	215
Diptera	Culicidae	*Anopheles*	1	0	0	0	1	0	0	0	0	0	0	0
Diptera	Unknown	Unknown	0	0	0	0	0	0	0	1	0	1	1	0
Diptera	Dolichopodiae	Unknown	0	0	0	0	0	0	0	0	0	7	0	0
Diptera	Dixidae	*Dixa*	0	0	0	0	2	0	0	0	0	0	0	0
Diptera	Dixidae	*Dixella*	1	0	0	0	1	0	0	0	0	0	0	0
Diptera	Empididae	*Chelifera*	0	0	0	0	21	1	15	1	2	1	4	0
Diptera	Empididae	*Clinocera*	0	0	0	0	0	0	3	0	0	0	0	0
Diptera	Empididae	*Hemerodromia*	0	0	1	0	25	27	12	15	10	15	26	5

Order	Family	Genus species	Control Site 1		Control Site 2		Restored Site 1		Restored Site 2		Restored Site 3		Restored Site 4	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Diptera	Empididae	Unknown	0	0	0	0	0	3	0	0	0	0	0	0
Diptera	Muscidae	*Limnophora*	0	0	0	0	2	0	7	0	0	0	0	0
Diptera	Psychodidae	*Pericoma*	0	0	0	0	1	0	1	0	7	1	0	0
Diptera	Simuliidae	*Simulium*	2	0	150	30	7318	108	9092	1343	4064	3241	639	177
Diptera	Stratiomyiidae	*Caloparyphus*	1	0	0	0	39	3	11	42	14	48	5	2
Diptera	Stratiomyiidae	*Euparyphus*	0	1	1	0	5	0	2	0	2	2	0	3
Diptera	Tabanidae	*Tabanus*	0	0	0	0	1	0	11	1	2	0	2	0
Diptera	Tipulidae	*Antocha*	0	0	0	0	1	0	4	0	0	0	0	0
Diptera	Tipulidae	*Dicranota*	0	0	0	0	32	0	29	0	0	0	1	0
Diptera	Tipulidae	*Hexatoma*	0	0	0	0	0	1	0	0	0	0	0	0
Diptera	Tipulidae	*Molophilus*	1	0	0	0	0	18	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula*	0	0	0	0	7	0	5	0	10	0	0	0
Diptera	Tipulidae	Unknown	1	0	0	0	0	0	0	0	0	0	0	0
Ephemeroptera	Baetidae	*Baetis*	1310	29	626	354	4328	393	6322	151	909	131	1402	40
Ephemeroptera	Baetidae	*Baetodes*	0	1	0	3	149	12	301	33	161	65	486	64
Ephemeroptera	Baetidae	*Callibaetis*	10	8	0	5	6	2	45	0	2	0	4	0
Ephemeroptera	Baetidae	*Cameleobaetidius*	0	0	0	1	0	0	0	0	0	0	1	0
Ephemeroptera	Caenidae	*Caenis*	30	4	91	6	185	9	108	0	0	0	0	0
Ephemeroptera	Ephemerellidae	*Serratella micheneri*	0	0	0	0	0	0	1	0	0	0	0	0
Ephemeroptera	Heptageniidae	*Epeorus*	0	0	0	0	1	0	0	0	0	0	0	0
Ephemeroptera	Heptageniidae	*Heptagenia*	0	0	0	0	0	0	1	0	0	0	0	0
Ephemeroptera	Leptohyphidae	***Leptohypes***	0	0	3	0	0	0	5	0	0	0	1	1
Ephemeroptera	Leptohyphidae	*Tricorythodes*	836	77	127	81	271	280	43	8	1	5	4	3
Ephemeroptera	Leptophebiidae	*Thraulodes*	71	35	584	275	47	10	7	0	0	0	0	0
Gastropoda	Ancylidae	*Ferrissia*	0	0	2	0	0	0	0	0	0	0	0	0
Gastropoda	Unknown	Unknown	0	0	0	0	0	0	19	0	0	0	0	0
Gastropoda	Hydrobiidae	*Pyrgulopsis simplex*	86	1	7	4	0	0	20	0	0	0	0	0
Gastropoda	Lymnaeidae	*Fossaria*	0	0	0	0	0	0	0	0	0	0	1	0
Gastropoda	Physidae	*Physa*	2	0	2	0	6	0	7	0	2	0	1	0
Gastropoda	Planorbidae	*Gyralus*	0	0	1	0	1	0	0	0	0	0	0	0
Hemiptera	Belostomatidae	*Belostoma*	0	0	0	0	3	0	0	0	0	0	0	0
Hemiptera	Gelastocoridae	*Gelastocoris*	0	0	0	0	0	0	0	0	0	0	1	0
Hemiptera	Gerridae	*Gerris*	0	0	0	0	0	0	0	0	0	0	1	0
Hemiptera	Gerridae	*Metrobates*	0	0	0	0	1	0	0	0	0	0	0	0
Hemiptera	Hebridae	*Hebrus*	0	0	0	0	4	0	0	0	0	0	0	0
Hemiptera	Naucoridae	*Ambrysus*	17	7	6	7	1	1	0	0	0	0	0	0
Hemiptera	Veliidae	*Mesovelia*	0	1	0	0	0	0	0	0	0	0	0	0

			Control Site 1		Control Site 2		Restored Site 1		Restored Site 2		Restored Site 3		Restored Site 4	
Order	Family	Genus species	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Hemiptera	Veliidae	*Microvelia*	0	1	0	0	2	0	1	0	0	0	0	0
Hemiptera	Veliidae	*Rhagovelia*	2	1	5	1	37	0	18	6	6	1	7	9
Lepidoptera	Pyralidae	*Petrophila*	8	3	48	21	34	89	70	6	166	15	97	16
Megaloptera	Corydalidae	*Corydalus*	6	1	13	21	16	53	58	4	8	29	8	8
Odonata	Anisoptera	Unknown	9	0	3	0	8	0	37	0	9	0	7	0
Odonata	Calopterygidae	*Hetareina*	3	0	1	2	5	0	16	0	2	0	1	0
Odonata	Coenagrionidae	Coenagrionidae	21	4	21	8	108	3	69	0	11	0	1	3
Odonata	Gomphidae	*Erpetogomphus*	4	9	1	8	3	4	8	1	0	2	2	0
Odonata	Libelluilidae	*Brechmorhaga*	1	0	0	0	1	0	19	0	0	0	10	0
Odonata	Libelluilidae	Unknown	0	5	0	1	0	1	0	6	0	0	0	0
Odonata	Unknown	Unknown	13	0	8	0	9	0	8	1	11	0	1	0
Ostracoda	Unknown	Unknown	157	0	272	0	391	2	25	0	3	0	2	0
Plecoptera	Capniidae	*Capnia*	0	0	0	0	0	0	4	0	0	0	4	0
Plecoptera	Nemouridae	*Malenka*	0	0	0	0	2	0	1	0	0	0	0	0
Plecoptera	Perlodidae	*Isoperla*	0	0	0	0	7	0	9	0	0	0	12	0
Trichoptera	Calomatoceridae	*Phylloicus*	3	0	1	0	5	1	0	0	0	0	0	0
Trichoptera	Glossosomatidae	*Culoptila/Protoptila*	0	0	4	0	0	0	1	0	0	0	0	1
Trichoptera	Helicopsychidae	*Helicopsyche*	3708	576	0	5	5	0	0	0	0	0	0	0
Trichoptera	Hydropsychidae	*Chuematopsyche*	0	0	25	15	38	7	16	0	0	0	10	14
Trichoptera	Hydropsychidae	*Hydropsyche*	167	21	209	312	140	170	134	0	1194	4	75	424
Trichoptera	Hydropsychidae	*Smicridea*	0	0	0	0	0	0	0	0	0	0	12	0
Trichoptera	Hydroptilidae	*Hydroptila*	0	0	0	1	47	8	46	0	0	1	3	2
Trichoptera	Hydroptilidae	Unknown	5	0	0	0	0	0	10	0	0	0	0	0
Trichoptera	Hydroptilidae	*Luecotrichia*	0	0	16	5	3	2	0	0	0	0	1	0
Trichoptera	Hydroptilidae	*Mayatrichia*	0	0	0	0	0	0	18	0	0	0	5	2
Trichoptera	Hydroptilidae	*Metrichia*	270	78	231	27	22	14	39	1	41	0	8	5
Trichoptera	Hydroptilidae	*Neotrichia*	0	0	0	0	1	0	0	0	0	0	2	0
Trichoptera	Hydroptilidae	*Oxytheira*	1	0	0	0	0	0	0	0	0	0	0	0
Trichoptera	Lepidostomitadae	*Lepidostoma*	0	0	1	0	0	0	0	0	0	0	0	0
Trichoptera	Leptoceridae	*Nectopsyche*	9	0	4	1	10	1	0	0	0	0	0	0
Trichoptera	Leptoceridae	*Oecetis*	0	0	0	0	5	0	0	0	0	0	0	0
Trichoptera	Limnephilidae	*Limnephilus*	0	0	0	0	2	0	27	0	0	0	0	0
Trichoptera	Odontoceridae	*Marilia flexuosa*	1	0	7	0	3	0	0	0	0	0	0	0
Trichoptera	Philopotamidae	*Chimarra*	0	0	8	5	43	31	65	0	3	2	8	0
Trichoptera	Philopotamidae	*Wormaldia*	0	0	0	0	12	3	4	0	93	1	1	0
Trichoptera	Polycentropidae	*Polycentropus/Cernotina*	0	0	7	5	101	0	23	1	0	0	1	0
Trichoptera	Polycentropidae	*Polyplectropus*	0	0	3	3	0	0	0	0	0	0	0	0

			Control Site 1		Control Site 2		Restored Site 1		Restored Site 2		Restored Site 3		Restored Site 4	
Order	Family	Genus species	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Trichoptera	Psychomyiidae	*Tinodes*	0	0	1	0	74	51	20	3	5	1	1	0
Trichoptera	Unknown	Unknown	0	0	4	1	1	1	1	0	1	0	0	0
Trombidiformes	Unknown	Unknown	6	11	11	26	57	39	44	33	7	1	22	2
Turbellaria	Tricladia	*Dugesia tigrina*	308	137	57	60	26	17	33	0	15	0	0	0