

Primary Research Paper

## Role of fish in structuring invertebrates on stromatolites in Cuatro Ciénegas, México

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### Abstract

Stromatolites, the dominant Precambrian life form, declined in the Phanerozoic to occur today in only a few sites. This decline has been attributed to evolution of metazoan grazers, but stromatolites in our study site, Cuatro Ciénegas, Coahuila, México, harbor diverse macroinvertebrates. Drawing on food chain theory, we hypothesized that fish predation on invertebrates controls invertebrate populations, allowing stromatolites to flourish in Cuatro Ciénegas. Our experiment used small mesh (1 mm) cages to exclude all but larval fishes, and larger (6.5 mm) cages to exclude all larger fishes (including the molluscivorous and omnivorous endemic polymorphic cichlid, *Herichthys minckleyi*), but allow access to all sizes of the abundant endemic pupfish, *Cyprinodon bifasciatus*. No effects of treatments on invertebrate densities were noted at 6 week, but significant effects were observed on specific taxonomic groups after 3 month. In absence of fishes, hydrobiidae snails and ceratopogonids increased 3- and 5-fold, respectively, and invertebrate assemblage composition varied among treatments. Algal biomass was not affected by treatments, but algal species composition appeared to change. Overall results suggest that fish assemblages structure invertebrate assemblages, and that fishes may also be factors in determining algal communities.

### Introduction

The decline of stromatolites, rock-like depositions of carbonates and sediments formed by algae (Lee, 1999) in the Middle Ordovician Period, roughly 570 million years ago, has been attributed to the evolution and diversification of metazoan grazers (Garrett, 1970; Awramik, 1971; Walter & Heys 1984). The hypothesis is that grazing by metazoans, along with possible bio-turbation, negatively affected both the algal

assemblages and environmental conditions necessary for stromatolite formation and growth. Experimental work demonstrating that grazers can disrupt microbial mats supports this hypothesis (Garrett, 1970), but it is principally based on the temporal correlation in the fossil record between metazoan diversification and stromatolite decline, along with observations that most modern stromatolites occur in “extreme” environments that limit metazoan diversity and abundance.

With discovery of stromatolites in “non-extreme” environments (most prominently in Cuatro Ciénegas) that also harbor diverse metazoans, alternative hypotheses have been posed to explain the global decline of stromatolites include changing sedimentological conditions (Pratt, 1982), changes in seawater carbonate saturation (Grotzinger, 1990) and competition for space with other algal forms (Moore & Burne, 1994).

Application of food web theory may increase understanding of the interactions of vertebrates, invertebrates and stromatolites. Food web theory predicts that in a two trophic level system, top-down effects of herbivory will more strongly limit standing biomass and algal growth than resources (Hairston et al., 1960; Fretwell, 1977; Oksanen et al., 1981), whereas resources limit primary production in a three level system, since herbivory is controlled by predation from top trophic levels. Recent food web studies have shown this to be a simplified view, with complex trophic interactions and resource limitation also being important, but this is still a useful heuristic framework (Polis & Winemiller, 1996). Studies have demonstrated that predators and herbivores can affect abundance and diversity of lower trophic levels in a variety of aquatic habitats (Power et al., 1985, 1988; Threlkeld, 1988; Power, 1990; Martin et al., 1992; Rosemond et al., 1993; Brönmark, 1994; Wootton, 1995; Marks et al., 2000), but no studies have yet addressed trophic interactions between vertebrates and invertebrates on stromatolites.

Modern stromatolites of Cuatro Ciénegas, in the Chihuahuan Desert, provide an opportunity for testing these interactions. Aquatic habitats of Cuatro Ciénegas support diverse but low densities of metazoan assemblages, particularly snails and insect larvae (Winsborough, 1990; Dinger et al., 2005), whereas fish diversity and abundances are high (Minckley, 1984). Two fishes commonly co-occur in high abundance with stromatolites: a large polymorphic cichlid, *Herichthys minckleyi*, and a small cyprinidontid pupfish, *Cyprinidon bifasciatus*.

In this study, we tested the hypothesis that fish control stromatolite invertebrate densities and assemblages. We predicted that the removal of all fishes would release invertebrates from fish predation, resulting in increased invertebrate abundances and decreased stromatolite growth.

We also predicted the 2 dominant fish species affect the invertebrates in different ways, depending on their diet. In particular, we hypothesized that the removal of the cichlid which sometimes consumes snails, would increase snail densities, whereas other invertebrates would be controlled by the cyprinidontid pupfish.

## Methods

### *Study site*

The study was conducted in Rio Mesquites, directly below the outflow of Poza Mojarral Este (lat 26° 55' N, long 102° 07' W) located in the Protected Area of Cuatro Ciénegas, Coahuila, México. The basin of Cuatro Ciénegas was declared a protected area in 1994, prior to which it was used for livestock grazing and fishing. Although areas of the basin are still used for grazing and agriculture, Poza Mojarral Este is located in a protected visitors center monitored daily by park staff. The outflow of Poza Mojarral Este form the Rio Mesquites – a clear, swiftly flowing stream ranging between 10–15 m wide and 50–80 cm deep.

The modern stromatolites of Rio Mesquites are laminated, benthic, microbial carbonate deposits caused by biological activity, where the microbial components incorporate carbonate into their extracellular material (Winsborough, 1990). Specifically, uptake of CO<sub>2</sub> through photosynthesis of the algal assemblage shifts the carbonate equilibrium to the point of supersaturation of calcium carbonate. The resulting precipitates are incorporated and bound into the matrix of the stromatolite algae. Bound and trapped precipitates lithify into the rock-like structures of stromatolites. In layman's terms, stromatolites are living rocks created by algae.

High densities of stromatolites ( $5.8 \pm 0.76$  per m<sup>2</sup>) shaped like oblong spheres (called oncoid stromatolites) with diameters ranging from 2–30 cm are found in the middle of the channel. We used stromatolites averaging 15 cm ( $\pm 0.192$  SE,  $n = 30$ ) in this experiment. The most important microbial mat components responsible for stromatolite growth in the Rio Mesquites are the green alga *Gongrosira calcifera* Krieger, the

cyanobacteria *Homoeothrix balearica* Bornet and Flahault and *Schizothrix lacustris* A. Brown, and a diverse assemblage of benthic diatoms (Winsborough, 1990). In this study, *Encyonema evergladium* Krammer, *Amphora katii* Selva (endemic to the Cuatro Ciénegas Basin), *Denticula kuetzingii* Grunow, *Mastogloia elliptica* (Agardh) Cleve, *Brachysira vitrea* (Grunow) Ross and *Encyonemopsis cesatii* (Rabenhorst) Krammer were the most abundant diatoms. These diatoms attach to the substrate with mucilage in the form of capsules, stalks, plaques and pads. This mucilage has been directly associated with carbonate precipitation (Winsborough, 2000).

Although 18 native fishes are found in the Cuatro Ciénegas basin (Minckley, 1984), only a few are found in association with oncoïd stromatolites at this site. The most prevalent fishes associated with stromatolites are the polymorphic cichlid *Herichthys minckleyi* and the pupfish *Cyprinodon bifasciatus*. *Herichthys minckleyi* has two morphologies that occur in about equal abundance in the study reach. One, with papilliform pharyngeal dentition primarily consumes algae, detritus and soft-body invertebrates. The other, with molariform pharyngeal dentition, can also eat native snails (Sage & Selander, 1975; Smith, 1982; Hulsey et al., 2001). Both *H. minckleyi* and *C. bifasciatus* have been observed "pecking" directly upon stromatolites, as well as using stromatolites as refuge and habitat (Winsborough, 1990, Swanson personal communication). Adult *H. minckleyi* range from 54 to 183 mm standard length (SL), whereas *C. bifasciatus* are much smaller and range from 12 to 30 mm SL.

#### *Experimental design*

The study was conducted from 20 June to 19 September 2000 in 20 caged enclosures (50 cm × 50 cm) constructed of PVC pipe framing with either Vexar mesh (6.35 mm) or fiberglass screen mesh (1 mm) enclosing the sides and bottoms. We used four experimental treatments: (1) large fish enclosure (excluding only *H. minckleyi* and other large fishes); (2) total fish enclosures (excluding all fishes except for larval and very small juveniles); and (3) and (4) cage controls for both fish enclosures. Vexar mesh excluded larger fishes (primarily *H. minckleyi*), and fiberglass screen mesh excluded

all fishes (except for larvae and very small juveniles). Cage controls, identical to full enclosures but open on one side, allowed access to all fishes and were used to assess cage effects. Five replicates of each treatment were randomly assigned to plots where stromatolites were occurring within main channel of the river. Cages were stocked with two oncoïd stromatolites (mean diameter of 15 cm) haphazardly picked from the channel stromatolites bed.

Cages were kept clean of detritus and algal growth by scrubbing with a nylon brush approximately every 2 days. Observations of fishes showed no reaction to cleaning procedures, and fish assemblages in open cages were similar to ambient conditions (Dinger, unpublished data).

Although a higher number of replicates would be ideal owing to naturally high levels of variability in aquatic ecosystems, the scarcity of stromatolites (locally dense, but scattered distribution) limited us in our ability to measure and manipulate them for this experiment.

#### *Experimental harvest and laboratory analyses*

##### *Collection*

One stromatolite was randomly selected from each cage and destructively sampled on 27 July 2000 (6 week) and again at a final harvest on 19 September 2000 (3 month). After removal, stromatolites were digitally photographed to estimate length and width (Adobe Photoshop version 5.5), while height was measured in the field with a ruler. The average diameter for each stromatolite was calculated and used in the geometric formula of a sphere to estimate surface area.

##### *Algal biomass and diatom assemblage*

A 10.16 cm<sup>2</sup> block was cut from the upper surface of each stromatolite and preserved in 10% formalin. The sample was used to determine ash-free dry mass (AFDM) using standard methods (Greenberg et al., 1992). Samples were dried at 60 °C to constant mass (about 48 h), weighed and then ashed for 1 h at 500 °C and reweighed to the nearest 0.1 mg.

Algal scrapes of the upper surfaces of each stromatolite were taken for analysis of the diatom species assemblage and preserved in 10% formalin. Samples were agitated, homogenized and an

aliquot taken. Aliquots were cleaned with hydrogen peroxide and HCl to oxidize the organic material and remove calcium carbonate. After cleaning, aliquots were mounted onto microscope slides and random fields of diatoms were enumerated and identified until 500 individuals had been counted to obtain relative abundance of individual species.

#### *Invertebrate assemblages and biomass*

Stromatolite invertebrates were collected by disaggregating each stromatolite into a 5 gallon bucket of water. Lighter sediments and most invertebrates were elutriated five times into a separate bucket. Larger portions of the disaggregated stromatolite were visually inspected for remaining invertebrates, particularly snails. The elutriate was then filtered through a 253  $\mu\text{m}$  mesh net and preserved in 95% ethanol for laboratory sorting.

Macroinvertebrates were sorted under 8 $\times$  magnification using a dissecting microscope. Specimens were identified to the lowest possible taxonomic level (usually genus) using keys for North American invertebrates (Needham & Westfall, 1954; Pennak, 1989; Thorp & Covich, 1991; Merritt & Cummins, 1996; Westfall & May, 1996; Wiggins, 1996). Subsamples of macroinvertebrates ( $n = 5\text{--}10$  for different taxa) were used to determine average ash free dry mass for common taxonomic groups, following the same procedure used for algal biomass.

#### **Statistical analyses**

Independent one-tailed  $t$ -tests were used to test for differences in invertebrate densities and algal biomass between fish enclosures and their respective controls (e.g. large fish enclosure and 3-sided control). One-tailed tests were based on the a priori hypothesis that invertebrate densities would increase in the absence of fish. Tests were conducted for total invertebrate biomass and numbers. Responses of individual species were tested for those present in over 50% of all replicates.

Differences in taxonomic composition among treatments were calculated using the Sorenson (Bray-Curtis) distance measure, which is robust for analyses of taxonomic data compared to Euclidean or other distance measures (Faith et al.,

1987; McCune & Mefford, 1999). Prior to computing dissimilarities, abundance values for each taxon were relativized to the maximum obtained for each taxon using:

$$b = x_{ij} / x \max_j$$

where  $b$  is the new value used in computing dissimilarities, and  $x \max_j$  is the highest value in the matrix for species  $j$ . This standardization equalizes the potential contributions of taxa to the overall dissimilarity, so that distance measures are not dominated by highly abundant taxa.

In order to test for significance of treatments on taxonomic composition, Sorenson distance measures were analyzed with the Multiple Response Permutation Procedure (MRPP) routine in PCORD (version 4.02). This is a non-parametric procedure to test for the difference between a priori treatment groups. MRPP has the advantage over discriminate analysis of not requiring assumptions about normality or homogeneity of the community data. In this test, the statistic  $A$  is a measure of effect size, where  $A = 1$  indicates that samples *within* a treatment group are identical but different from other treatments, and an  $A = 0$  indicates that samples are heterogeneous *between* treatments. In other words, an  $A$  near 1 indicates strong treatment grouping, whereas an  $A$  near 0 indicates weak treatment grouping. If significant grouping occurred, MRPP was used in pairwise comparisons to determine which specific treatment groups were significant and which were not.

Upon determination of significant groupings, we used Non-metric multidimensional scaling (NMDS ordination) on the significant groups to graphically represent the invertebrate and diatom assemblage differences. NMDS was used because it does the best possible job of maintaining the relationship between samples in low-dimensional pictures (Clarke, 1993). The minimum stress (0–100) in each dimension was examined and determined that a three-dimensional plot provided an adequate summary of the pattern of dissimilarities.

To assist in interpretation of the NMDS ordination, we used the biplot routine in PCORD (version 4.02) to correlate a secondary matrix with the configuration of samples in the NMDS. The variables used in the secondary matrix were: species richness, evenness, Simpson's  $D$ , Shannon's  $H$ ,

snail diversity, dipteran diversity, ephemeroptera diversity, predator diversity (mainly odonates), and other diversity (total diversity of all other invertebrates). These variables were used only to assist in interpreting the configuration, so there is no problem with any lack of independence with these variables.

## Results

### *Invertebrate densities*

Experimental stromatolites provided habitat for 17 invertebrate taxa. Commonly occurring invertebrate taxa included midges (Chironomidae), amphipods (*Hyalella* sp.), caddisflies (*Cernotina* sp.), biting midges (Ceratapogonidae), mayflies (*Caenis* sp.) and the hydrobiidae snails, *Mexithauma quadripallium* and *Nymphophilus minckleyi*.

Other less abundant taxa included riffle beetles (Coleoptera:Elmidae), *Dorocordulia* sp. (Anisoptera: Corduliidae), *Macromia caderita* (Anisoptera: Corduliidae) and *Argia* sp. (Zygoptera:Coenagrionidae). Rare taxa were *Hexatoma* (Diptera:Tipulidae), *Mexipygrus carranzae* (Gastropoda: Hydrobiidae), *Dugesia* sp. (Turbellaria), ostracods, oligochaetes, and water mites (Acarina). Many of these were more commonly associated with the surrounding sediments, but were occasionally found on stromatolites.

At the 6 week midpoint harvest, exclusion of fishes did not significantly affect either total stromatolite invertebrate densities, or densities of any specific taxonomic group (i.e. snails, amphipods, etc.) (Table 1). After three months, there was no significant effect of fish exclusion on total invertebrate densities (Fig. 1, Table 1) or on total invertebrate biomass (Fig. 1, Table 1), although the trend was for the highest total biomasses to be in treatments that excluded fishes.

In contrast to total invertebrate density, there were strong, significant effects on specific groups. Snail density (*M. quadripallium* and *N. minckleyi*) tripled in treatments that removed the large, endemic cichlid, *H. minckleyi* (and pupfish were still present) compared to its control (Fig. 2, Table 1). Snail densities were also higher in total fish enclosures relative to controls, but lower than when only large fish were excluded. Densities

of biting midges, *Culicoides* sp. and *Bezzia* sp. (Diptera: Ceratapogonidae) increased five fold in the absence of all fish predation (Fig. 2, Table 1) compared to the cage control. The two most numerically dominant taxa, (*Hyalella* sp. and Chironomidae) did not respond to experimental treatments (Table 1).

### *Invertebrate assemblage and biomass*

The MRPP analysis showed significant differences in taxonomic composition among treatments ( $A=0.066$ ,  $p=0.015$ ). In pairwise comparisons, the strongest effect was between the total fish exclusion and the total fish cage control (Table 2). The differences in taxonomic composition of these two treatments are demonstrated in the NMDS ordination (Fig. 3). The overlaid biplots show that the exclusion of all fishes are associated with increasing species richness, snail diversity, Shannon's H, Simpson's D, and evenness. In this configuration, the x-axis explains 61.5% of the variance, and the y-axis explains an additional 20%.

There were also significant differences in invertebrate assemblages between exclusion of all fish and exclusion of large fish, but there were no significant differences between large fish enclosures and large fish cage controls (Table 2).

Furthermore, changes in invertebrate assemblages were reflected in changes in percent relative biomass of species across treatments (Fig. 4). Biomass was measured for the 8 most prevalent species, which constitute approximately 95% of total invertebrate biomass. The relative biomass of snails (*M. quadripallium* and *N. minckleyi*) on cage control stromatolites were approximately 40% but increased to 70.3% in the absence of *H. minckleyi* and 76.9% in the absence of all fish. In contrast, the relative biomass of chironomids and amphipods (*Hyalella* sp.) decreased in the absence of fish predation.

### *Algal biomass and diatom species assemblage*

Fish exclusion did not alter algal biomass after three months (Fig. 2). One-way *t*-test analysis on the effects of removing large ( $p=0.763$ ) and all fishes ( $p=0.867$ ) were both nonsignificant. Macroscopic examination of stromatolite surfaces indicated a possible shift in species composition

Table 1. Means and standard errors for dominate invertebrates and total invertebrate densities and biomass

Taxonomic Groups	6 weeks				3 Months			
	Large Fish Exclosure	Large Fish Cage Control	Total Fish Exclosure	Total Fish Cage Control	Large Fish Exclosure	Large Fish Cage Control	Total Fish Exclosure	Total Fish Cage Control
<i>Caenis</i>	39.5 (30.9)	9.2 (3.8)	18.9 (12.3)	37.5 (7.5)	48.0 (38.7)	12.8 (4.4)	29.2 (19.8)	12.9 (6.2)
<i>Ceratonia</i>	87.9 (24.0)	95.6 (27.7)	59.9 (17.9)	55.8 (27.1)	169.9 (32.0)	164.1 (36.8)	19.0 (10.2)	76.1 (21.5)
Chironomidae	1337.0 (306.2)	1384.4 (288.4)	809.3 (317.8)	1475.8 (459.9)	2384.4 (418.3)	2370.8 (650.0)	1281.0 (276.3)	1903.6 (294.5)
<i>Culicoides</i>	51.1 (11.1)	28.3 (10.9)	70.2 (18.7)	37.9 (16.9)	85.8 (32.9)	38.6 (13.9)	<b>276.9 (56.9)</b>	<b>54.8 (25.5)</b>
<i>Hyalella</i>	660.9 (279.1)	989.8 (601.3)	2043.3 (1059.6)	1801.0 (747.8)	749.9 (432.6)	776.0 (514.5)	221.4 (86.4)	534.8 (222.3)
Hydrobiidae	162.5 (62.8)	92.2 (38.4)	66.1 (20.1)	87.8 (29.2)	313.1 (125.9)	95.9 (39.2)	<b>188.6 (42.8)</b>	<b>91.7 (26.9)</b>
Total Invertebrate Density	2454.3 (342.9)	2667.2 (610.6)	3146.2 (1012.2)	3562.1 (700.4)	3885.9 (649.1)	3514.3 (673.5)	2066.6 (241.4)	2696.5 (400.3)
Total Invertebrate Biomass	NA	NA	NA	NA	0.272 (0.08)	0.140 (0.03)	0.164 (0.03)	0.107 (0.02)

Significant comparisons using *t*-tests (df= 8) of exclosures to respective cage controls are presented in **bold** at 0.05 level. NA indicates data not available.

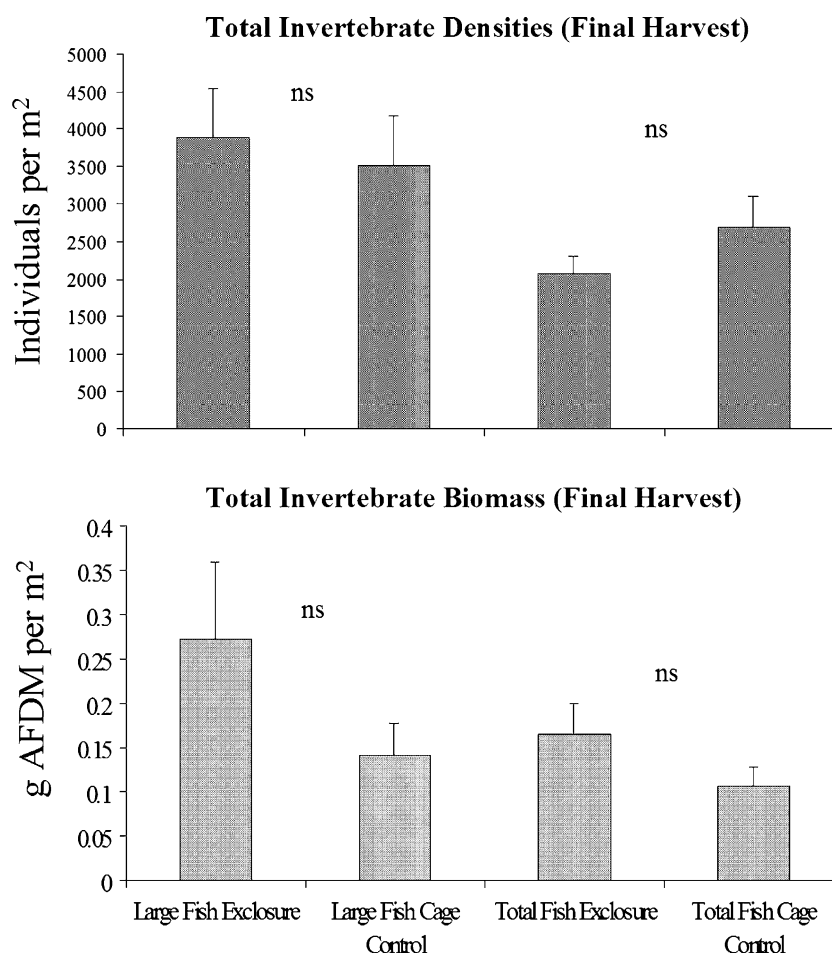


Figure 1. Mean densities with standard errors of total invertebrate densities at final harvest (top) and total invertebrate biomass for final harvest (bottom). ns indicate *t*-test was non-significant.

(Fig. 5), although NMDS ordination and *t*-tests between treatments on individual diatom species did not show any significant differences or patterns between treatments.

## Discussion

As predicted, the invertebrate assemblages responded differently to varying fish assemblages. Although the most numerically dominant invertebrates (*Hyalella* sp. and Chironomidae larvae) did not respond, several select taxa did. The 3-fold increase in snail densities in the absence of cichlid predation matched our predictions. We did, however, expect to see a similar increase in snail densities with the absence of all fish – and while

density increased, the effect size was not as large. Surprisingly, changes in invertebrate assemblages did not cascade to the algal biomass, but may have resulted in a shifting algal species assemblage (See Fig. 5).

General trophic models predict that fish removal would increase invertebrate biomass and decrease algal biomass ( Hairston et al., 1960; Fretwell, 1977) but experimental manipulations have had mixed results. Evidence from many freshwater systems support the trophic cascade model in fish–zooplankton–phytoplankton food webs although strength of the response on algal biomass has varied from “strong” to “weak” (see Brett & Goldman, 1996 for meta-analysis). Similarly, in stream ecosystems top predators have been shown to have strong effects in some systems,

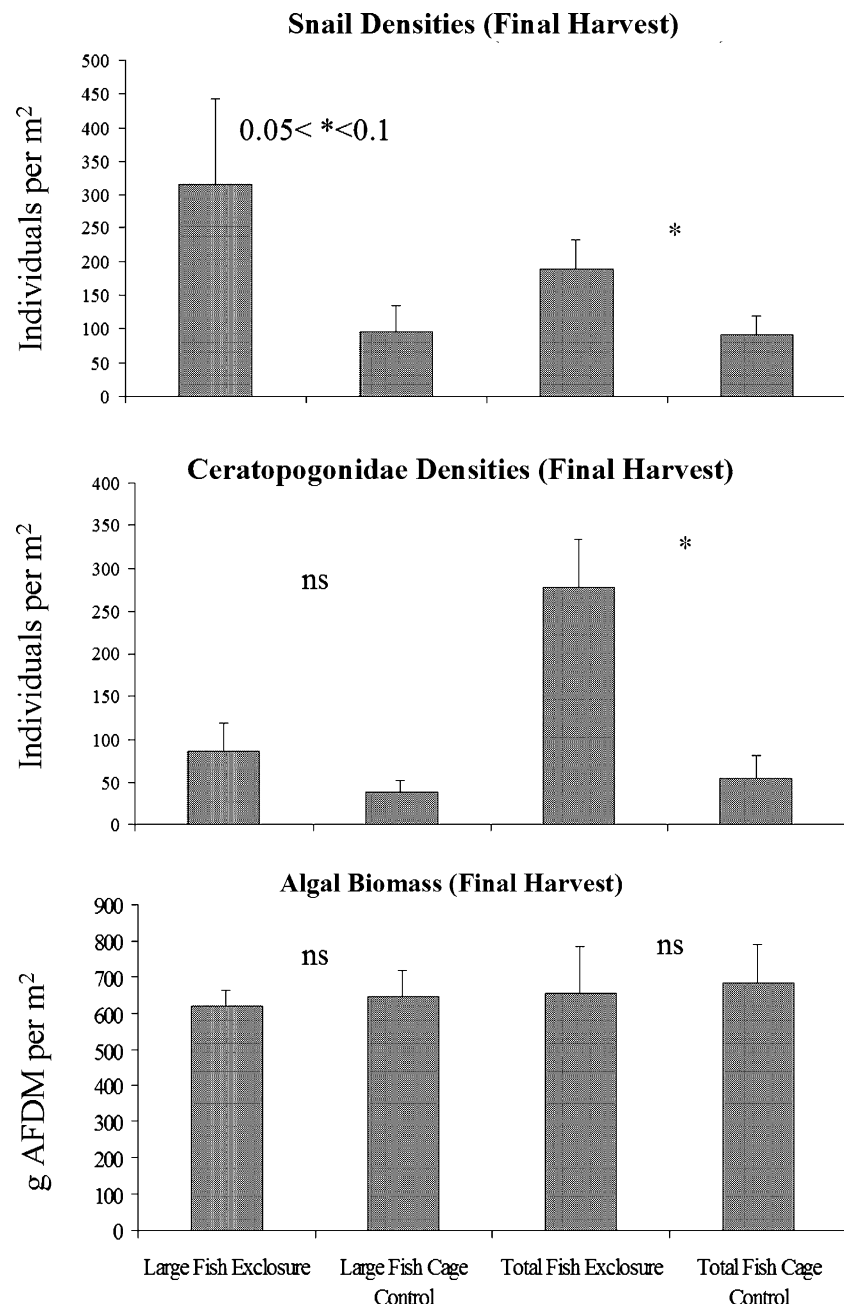


Figure 2. Mean densities with standard errors of Hydrobiidae snails (top), *Culicoides* (middle) and algal biomass (bottom) for final harvest. ns indicate *t*-test was non-significant. \* indicates *t*-test significant at 0.05 level.  $0.05 > * > 0.1$  indicates marginal significance.

but little to no effects in others. Fish effects depend on a variety of other factors, especially when food web linkages are between pelagic fish and benthic invertebrates and algae. For instance, Marks et al. (2000) demonstrated that importance of fish

predation is dependent on functional food chain length, which is affected by disturbance (flooding), but also that fish effects on herbivores are influenced by specific herbivore habits (i.e., highly mobile grazers versus restricted mobility grazers).



Table 2. Results of MRPP pairwise comparisons testing for significant differences between the groups of treatments

Treatments Compared	A Statistic	p value
Large Fish Enclosure/Large Fish Cage Controls	-0.035	0.87
Total Fish Enclosure/Total Fish Cage Controls	0.128	0.004
Large Fish Enclosure/Total Fish Enclosure	0.052	0.05

Fish predation effects are also dependent upon behavior of grazers, which can change in the presence or absence of predation (McIntosh & Townsend, 1996).

#### Effects on invertebrates

Exclusion of fish did not increase invertebrate densities as predicted, in part because the two most numerically dominant invertebrates (Chironomidae and *Hyalella* sp.) did not respond to experimental treatments. Fish diet data reveals that these dominant invertebrates are a minor food item for *H. minckleyi* in comparison to detritus and algae (Smith, 1982; Hulsey, 2001). The lack of an effect on these may be due to their

specific habitats and availability of natural refuges. *Hyalella* tend to congregate in the lithified crevices on the uneven surfaces of stromatolites, which may provide an excellent refuge from fish predation. Chironomids, additionally, form silken tubes that are enmeshed within the algal matrix of the stromatolite surface which also may provide a refuge from predation. Other studies of invertebrates with a source of refuge have shown no fish effects (Marks et al., 2000). With these natural refuges, fish predation upon these taxa may be minimal even with normally occurring high densities of fish.

Fish affected other invertebrate species, but the effect depended upon which fish species was excluded. In treatments that removed large *H. minckleyi*, snail density increased, but the magnitude and significance depended also upon the presence or absence of the smaller pupfish, *C. bifasciatus*. When *C. bifasciatus* were excluded along with *H. minckleyi*, the snail density only doubled, but it tripled when only *H. minckleyi* was excluded. Increases in snail densities with the exclusion of large *H. minckleyi* can be attributed to release from predation pressures exerted by the molariform phenotype of *H. minckleyi*, which eats *M. quadripallium*, and to a lesser degree *N. minckleyi* (Smith, 1982; Hulsey, 2001).

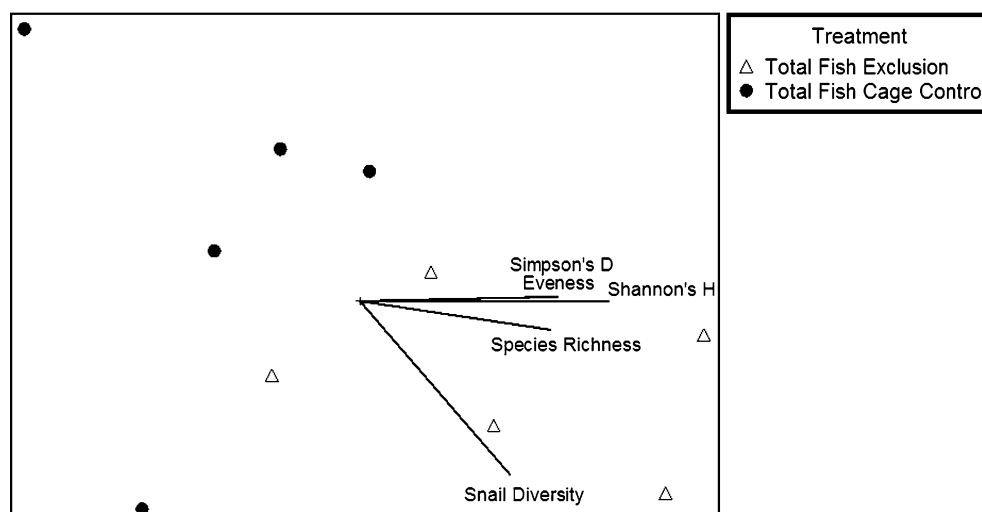


Figure 3. Non-Metric Multidimensional Scaling Ordination (NMDS) of invertebrate assemblages from stromatolites after three months (19 September 2000). Lines from centroids are biplots of variables with correlation values over 0.5. Note grouping of treatments, and that cages with total fish exclusion are associated with higher overall diversity (Simpson's D, Shannon's H, Evenness, and species richness) and higher snail diversity.

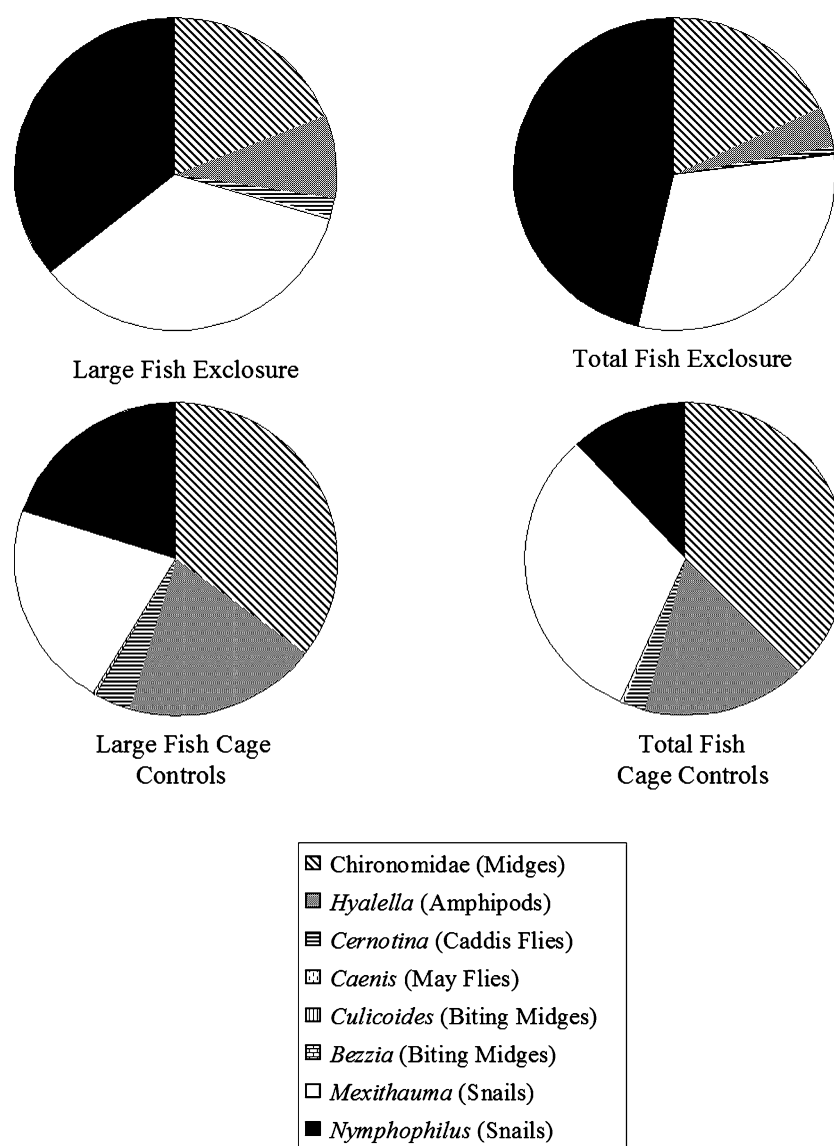


Figure 4. Mean relative biomass of invertebrates across experimental treatments after three months (19 September 2000). Note that only the eight most abundant invertebrates are included since there were not enough specimens for all invertebrate taxa. These taxa represent over 97% of the relative abundance, and over 99% of the total biomass.

The smaller effect on snail densities with complete fish exclusion may be due to negative interactions between the snails and other invertebrates (released from predation with pupfish removal). For example, our data show a five-fold increase in biting midges (*Ceratopogonidae*) in pupfish exclosures. *Ceratopogonidae* larvae are predators and could possibly be preying either upon snail eggs, or on the snails directly. Hence, the reduced

response of snail densities in the absence of all fish could be a balance between the positive effects of release from fish predation and negative effects of increases in other invertebrates due to increased predation or competition.

Although fish did not affect invertebrate biomass, they did change invertebrate species assemblages, which may be responsible for changing algal species composition. One of the most



*Figure 5.* Photographs of representative stromatolites upon harvest (19 September 2000). Stromatolites from fish exclusion treatments were noticeably gray-green (Top), while stromatolites from open treatments were generally olive-green (Bottom).

important of these changes may be the increase in snail densities. Although snails are a numerically small component of the invertebrate assemblage on stromatolites (2.6% on cage control stromatolites) their importance is highlighted by their relative biomass (40.2% on cage control stromatolites). Snail grazing pressure on stromatolites is possibly an important component structuring algal assemblages of oncoid stromatolites.

Lack of any effect upon any specific invertebrate taxon at the six week harvest, as well as significant effects by the end of the 3 month period suggest that a longer experiment may result in larger differences between treatments.

#### *Effects on algae*

Exclusion of fish did not result in any measurable effect on algal biomass. Lack of a detectable trophic cascade in this experiment may be the result of several factors.

First, oncoid stromatolite growth is slow (2–3 mm of growth per year [Winsborough, 1990]). Other food web manipulations of predator densities in streams have shown trophic cascades within three weeks to three months (Power et al., 1988; Threlkeld, 1988; Power, 1990; Marks et al., 2000). Given slow growth of stromatolites, detecting changes in algal biomass may require longer-term experiments that take into consideration environmental and seasonal variables influencing the composition of algal assemblages.

Second, both fishes (*H. minckleyi* and *C. bifasciatus*) “peck” at the outer layers of algae. Although the fish may be searching for invertebrates within the algal matrix, they may also be directly consuming algae. Hence, while fish removal may increase invertebrate grazing pressure, this may be offset by reduced fish herbivory on the algae. In prior studies where top consumers are omnivorous, trophic cascades have not been observed (Pringle & Hamazaki, 1998; Usio, 2000). While the extent of pupfish and the cichlid omnivory on stromatolites is not known, dietary studies of *H. minckleyi* have revealed a wide range of stomach contents including algae, calcite, snails, and other invertebrates (Smith, 1982; Hulsey, 2001). Simultaneous direct and indirect effects of fishes on intermediate consumers and primary producers may decouple a trophic cascade.

Third, stromatolite growth may be primarily controlled by resources, as well as top-down predation. This is because stromatolite flora depends on low nutrient concentrations that restrict the growth of weed algae and other aquatic plants that would otherwise overgrow the stromatolites. The waters of Cuatro Ciénegas are limited in nutrients with concentrations of PO<sub>4</sub> below detection limits (below 0.05 µg/l) (Kloepfel, 2002). Previous food web studies have shown that trophic levels are simultaneously controlled by both top-down and bottom-up effects (Power, 1992; Rosemond et al., 1993; Forrester et al., 1999; Marks et al., 2000). Hence, in Cuatro Ciénegas relative strength of bottom-up limitation on algae may be stronger than top-down herbivory.

Lastly, species composition of the surface of the algal matrix of stromatolites may be shifting. Macroscopic visual observation shows that the color and texture of the stromatolites differed depending on the treatments. In treatments that excluded fish, the algae is dull gray-green, slimy, and mucilaginous suggesting the presence of a surface coating of diatoms and the copious extracellular mucilage they produce. In contrast, the stromatolites in ambient conditions are an olive-green and lack any mucilaginous texture, an appearance more characteristic of fresh colonies of cyanobacteria. These composition shifts would not be detected by analyses of diatoms.

#### **Conclusion – Implications for stromatolite assemblages**

Although the data supporting a shift in algal species composition in this experiment is preliminary, it warrants additional research. Because stromatolite morphology and growth depend on specific types of algae that can incorporate calcium carbonate into their structure, any shift in algal species may pose a potential threat to stromatolites. If grazing pressures shift stromatolite algal composition to grazer resistant growth forms that are not conducive to the precipitation and binding of calcite, modern stromatolite growth would stop and become substrate for conventional algal growth. The possible fragility of stromatolite formation is highlighted by previously recorded cessation of stromatolite formation in Little Conestoga Creek,

Pennsylvania (Golubić & Fisher, 1975). A shift in the pH due to acid pollution halted the deposition of calcium carbonate, and formation of stromatolites stopped. Just as stromatolites depend on certain chemical conditions, they may also be dependent on the entire floral and faunal communities of their ecosystem.

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